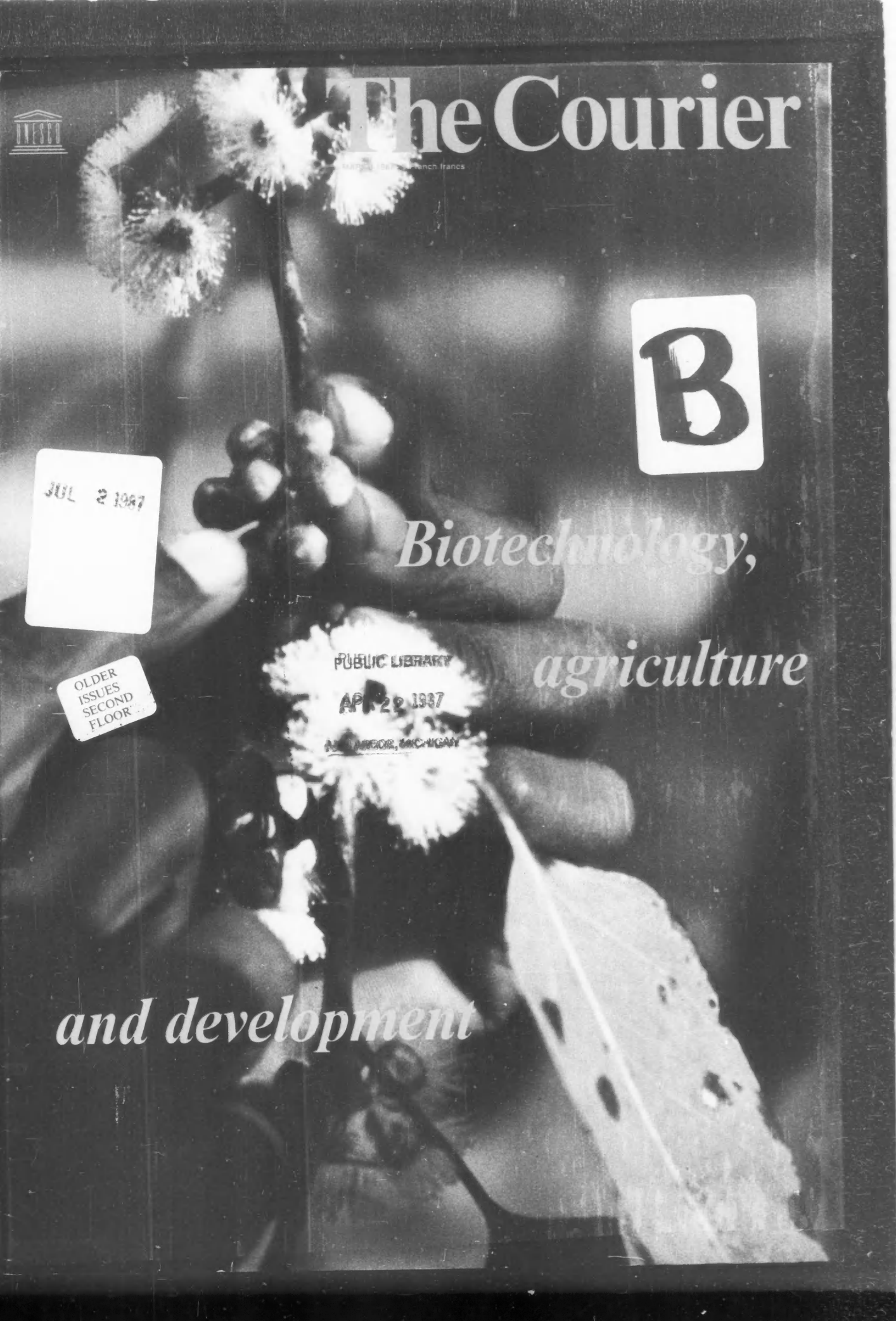
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A time to live...

Photo Bernard Wolff/UNICEF

Jumping for joy

Sierra Leone is a country on the west coast of Africa with a population of some 3,600,000. It takes its name ("Lion mountain") from that given by the Portuguese explorer Pedro da Sintra around 1460 to the peninsula which is the site of Freetown, the country's capital. In 1787 a settlement for freed slaves was established on land where Freetown now stands. In 1961 Sierra Leone achieved independence, and ten years later became a republic. Some 65 per cent of the work force is occupied in agriculture, with rice as the main food crop. Sierra Leone is the world's sixth largest producer of diamonds. Above, body bent back and almost obscuring the ball, a boy throws himself into a game of soccer in a Freetown street.

Editorial

In the 1960s and 1970s, the development of high-yield cereal varieties combined with the use of pesticides, irrigation and fertilizer brought a Green Revolution to some—but not all—parts of the Third World. This issue of the *Unesco Courier,* which is largely devoted to the application of new scientific techniques to agriculture, enquires into the extent to which the Green Revolution is likely to be followed by a "Biotechnological Revolution" which may help developing countries to solve some of their food production problems.

Although the term *biotechnology* to denote the use of the biochemical and genetic capacities of living organisms for practical purposes is fairly new, man has been engaged in "biotechnological" activities since very early times. Fermentation and the improvement of useful plant and animal varieties by cross-breeding are but two examples. The new biotechnology, however, differs from these time-honoured practices in that it uses genetic engineering and techniques of fusing cells of different organisms to surmount previously impassable barriers between species. Genetic engineering (or gene-splicing), which involves the direct transfer of genes—"those tiny command posts of heredity that tell living cells whether they will become bacteria, toads or men"—into the cells of different species has been described as the "most powerful and awesome skill acquired by man since the splitting of the atom."

In the first part of this issue we look closely at some of these new biotechnologies: how they work; how they are currently being used in different parts of the world and to what effect; the latest trends in this field where changes occur quickly and possibilities are vast. While our contributors focus mainly on the direct applications of biotechnologies to agriculture in the developing world, they also note present and potential uses in energy production, human and animal medicine and the management of certain environmental problems.

The second part of the issue asks broader questions. How can the new biotechnologies be best harnessed to development in different social, economic and cultural contexts? Will they be a panacea or, contrariwise, are they likely to aggravate existing disparities between developing countries and those of the technologically advanced world? The new biotechnologies and especially those applied to plants, put great possibilities into the hands of those who control them. How should this power best be exercised? How should access to the fruits of research based on plant genetic resources originating in the developing world be equitably organized?

Unesco, which is engaged in worldwide efforts to strengthen rural development through training in the biological and agrobiological sciences, in applied microbiology and in biotechnology (see article page 27), is closely interested in the above issues as part of its basic commitment to promote the use of science and technology for the benefit of all humanity. The complex nature of the problems and some possible approaches and solutions are traced by Dr. Albert Sasson in the article which forms the conclusion of this issue.

Editor-in-chief: Edouard Glissant

March 1987

40th year

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by Jacques C. Senez

The new biotechnologies

Promise and performance

SINCE the beginnings of civilization, man has been a biotechnologist, taking advantage of the activities of micro-organisms of whose very existence he was unaware, to produce foodstuffs and fermented drinks. Over the centuries, the practices by which he did this gradually developed, in a makeshift, empirical fashion, to attain a high degree of perfection. Yet biotechnology proper, in the sense of the scientific use of biological principles for practical purposes, only emerged at the end of the last century with the birth of microbiology and its early application to industrial fermentation processes.

Since the Second World War, biology has made prodigious progress. In just a few years the basic mechanisms of life and heredity at the molecular level have been unveiled, thus opening up limitless horizons. Some of these prospects, in particular that of the development of genetic engineering (see article page 13) with its connotations of man the creator, captured the imagination and fired the enthusiasm of the general public.

The transfer of genetic material between organisms as widely different as bacteria, plants, animals and man gave rise to great hopes, some of which, such as the production of human insulin by bacteria recombined *in vitro*, have already become reality. Today, these methods are on the verge of finding new applications of considerable economic and social importance in the field of agriculture. It would be wrong, however, to think that the prospects for biotechnology are limited to the field of genetic engineering. Recent advances in fundamental knowledge and techniques in the physiology of cells, biochemistry, enzyme catalysis and bioengineering are just as promising.

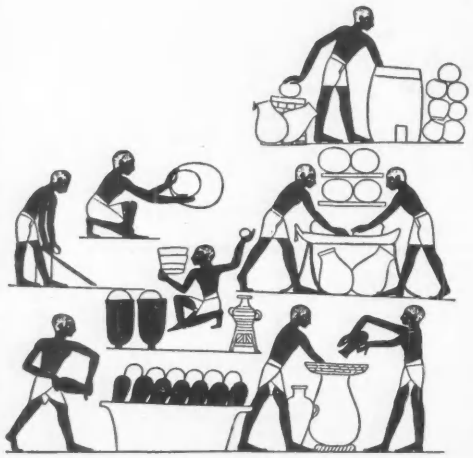
It is generally thought that there is a great future for biotechnology in the developing countries, particularly in its applications to agriculture. These hopes are well founded, but it should not be forgotten that progress involves potential dangers against which all possible preventive measures should be taken.

The first major achievement of agricultural biotechnology was the "Green Revolution', whose ambitious objectives have been largely achieved (see box page 7). Thanks to the Green Revolution India, Bangladesh and several other Third World countries have achieved self-sufficiency in food. This was a true success, but it brought in its train a number of unforeseen social consequences. The farming of high-yield cereals requires considerable investment in fertilizers, pesticides and irrigation which many small farmers were unable to make. As a result many of them had their fields taken over by the large landowners and were forced to move to the cities and swell the ranks of the sub-proletariat.

The recent collapse in the world price of sugar is another example of a social and economic backlash due to biotechnology. Due largely to the production of iso-glucose in the United States, this sugar price collapse has spelled ruin to a number of tropical countries whose economies are based on sugar-cane.

Fortunately, not all biotechnology applications entail problems such as these. However, there is a danger that some of them will further increase rather than diminish Third World dependence on the richest and most scientifically advanced countries (see article page 29).

Bearing this in mind, the developing countries must concentrate their efforts on programmes which are both of direct interest to them and which can be implemented immediately within the limitations of their financial and economic resources. Many such opportunities are open to them in agriculture in which two avenues in particular beckon: that of **primary production,** where there are possibilities in the field of plant improvement and nitrogen fixation, and that of **bioconversion** of agricultural products and wastes into energy and food resources.



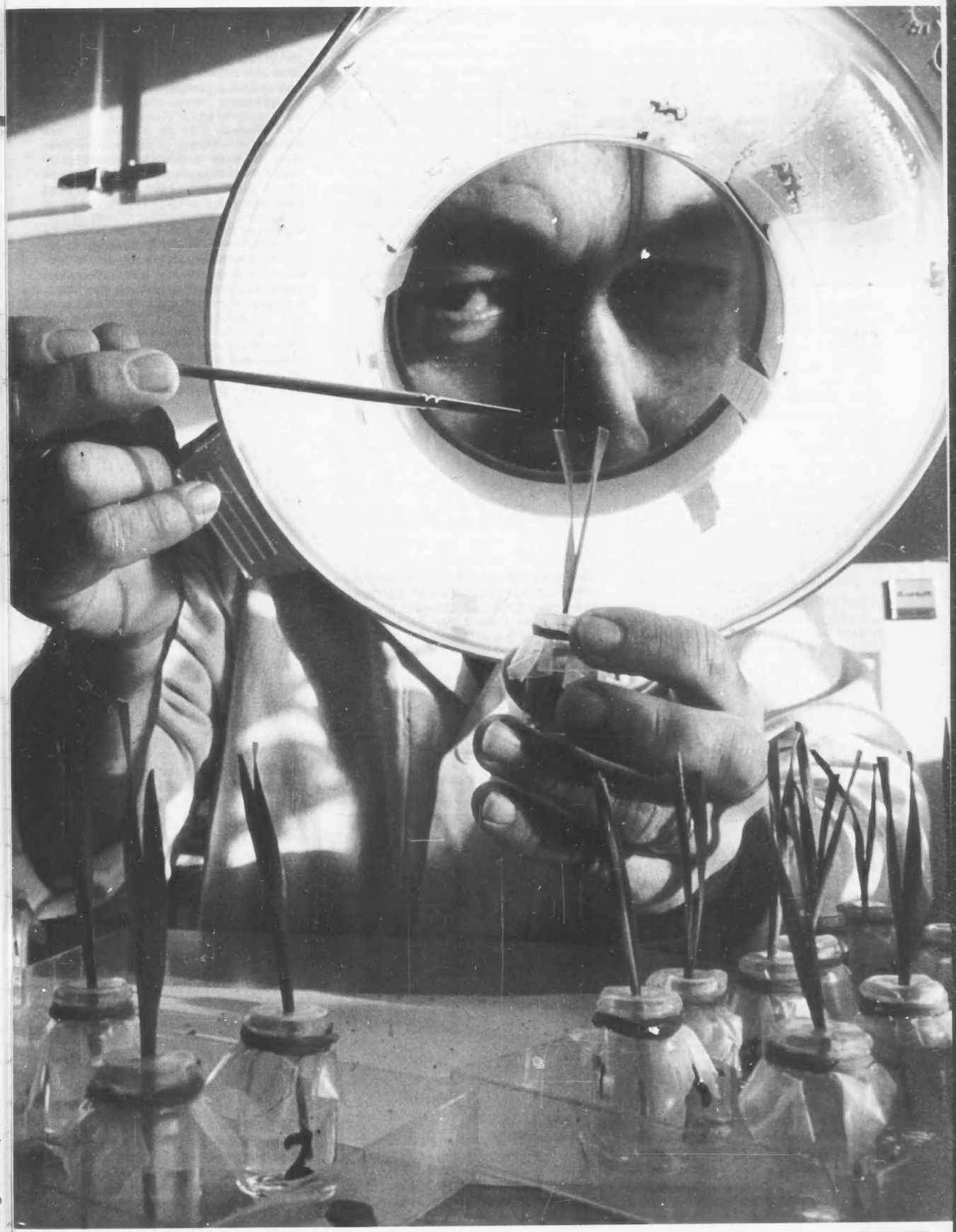
Egyptian bakers and brewers of 3,500 years ago are shown at work in this scene from a Theban tomb. "Biotechnological" processes such as microbial fermentations have been used for thousands of years to produce beverages and foods such as beer and cheese.

Drawing taken from *A History of Technology*
© Oxford University Press

New techniques for better plants

Plant improvement by the traditional methods of selection and cross-breeding is as old as agriculture itself. Thanks to recent advances in knowledge of the genetics and physiology of plants these methods have been refined and will long continue to produce very important results. During the past thirty years, for example, the yield of maize has increased from 12 to 62 quintals per hectare, while that of wheat has grown on average by one quintal per hectare per year. Similar progress has been made with rice, the second most important of the great cereals in worldwide use. Today, the International Rice Research Institute (IRRI), set up in the Philippines in 1962, has a collection of 60,000 varieties of rice (see the *Unesco Courier,* December 1984).

Photo © Alain Le Toquin, Paris



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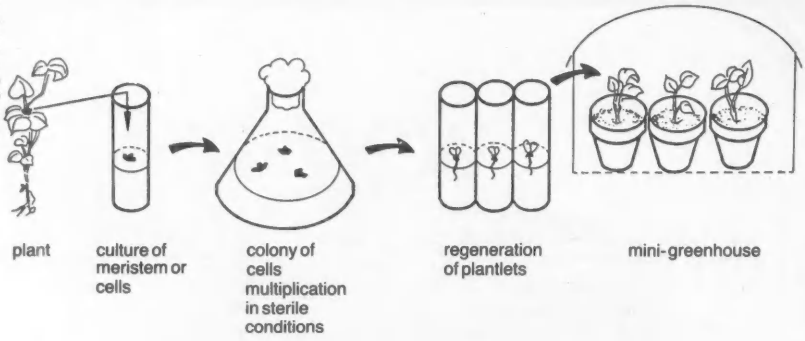


Drawing © Biofutur, Paris

Drawing shows in highly simplified form one of the techniques used in modern biotechnology for experimentation under controlled conditions with plant cells, tissues and organs and for vegetative (i.e. non-sexual) propagation of plants in sterile laboratory conditions. The sterilized plant material which is cultured in the nutrient medium may be a meristem (see drawing at bottom of page), or some other piece of plant tissue (see photo story pages 8-9), or a protoplast, a plant cell whose outer walls have been removed (see page 10). From this organ, tissue, or protoplast, a proliferating clump of disorganized tissue called a callus can be obtained. From this it is possible to regenerate whole intact plants, and to produce many genetically identical copies, known as clones, in a relatively short time. A one-cubic-centimetre culture may contain one million cells each carrying the potential of becoming an entire new plant. By selecting cells with certain properties, the process of breeding new varieties of disease-resistant, stress-tolerant crops, trees or flowers can be greatly accelerated.

In addition to improving yield, the main purpose of selection is to obtain new varieties which are resistant to parasites and to bacterial and viral diseases. In recent years a number of new techniques have made their appearance, some of which are already in use while others are still at the laboratory stage. One of their main aims is to reduce considerably the time needed for a new variety to be put on the market and brought into large-scale cultivation. Using classic methods the lead time required to achieve this is of the order of ten years, whereas, given the capacity for adaptation of the phytopathogenic agents (the bacteria, viruses, etc., that cause plant disease), the useful life of a new variety is estimated to be a mere five years.

Another advantage of certain recently evolved techniques is that they make it possible to cross-breed species that are too far apart for normal sexual reproduction, thus opening the way for the creation of entirely new plant varieties.

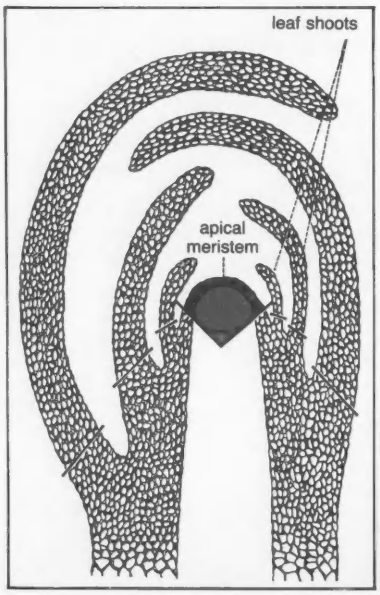
The first major successes were achieved by means of **vegetative hybridization** of cereal seedlings. This method, which consists of cross-breeding between plants by the elimination of self-fertilization, is comparatively easy in the case of allogamous cereals, such as maize, in which the male organs are separated from the female organs and can thus be manually eliminated before fertilization has taken place. It is more difficult with autogamous plants, such as wheat, tomatoes, soya and lupin, in which the male and the female organs are contained in close proximity within the flower. Today, this difficulty has been overcome by the discovery of chemical compounds which render the pollen sterile.

Many varieties of hybrid cereals and other plants are now on the market. Generally speaking, fields should be sown with first generation hybrid seed. Hybrid seed usually tends to degenerate and must be renewed annually. At all events, the world market for hybrid seed is growing rapidly and, according to a recent estimate, will attain a value of $20,000 million by the year 2000.

Other techniques now being developed are more distant in prospect yet just as promising. One of these is *in vitro* **vegetative propagation,** or micro-propagation, by the culture of the meristem or other plant tissues. Meristem is the name given to a grouping of embryonic cells situated at the tip of the plant stem (see drawing below). Cultivated in aseptic conditions on a solid, nutritive culture medium, these cells proliferate producing a callus which can be divided and reproduced many times. Treated with plant hormones (auxins, cytokins and gibberelins), the calluses differentiate into plantlets having all the properties of the original plant.

By this means, in a period of eight months, 2,000 million identical tubers, spread over an area of 40 hectares, were obtained from a single potato tuber derived from a meristem, that is a rate of propagation 100,000 times greater than that of sexual reproduction. A further advantage is that plants obtained from meristems are free of pathogenic contaminants, in particular of viruses, which means that it is possible to regenerate stock threatened with extinction due to diseases that cannot be treated in any other way.

The apical meristem is a tiny mass of cells where growth takes place at the tip of a plant stem. It plays a particularly important role in plant propagation because it remains healthy even when the rest of the plant is infected with a virus.* In vitro *culture of the meristem of a diseased specimen makes it possible to generate a new, healthy plant, and allows the rapid production of virus-free planting materials. Below, sectional drawing of a plant bud shows the apical meristem at centre, protected by enfolding leaf shoots. Meristem culture calls for particular care in the choice of culture conditions and nutritive media.



Drawing © La Recherche, Paris

Tropical agriculture has much to gain from micro-propagation. For example, a single oil palm regenerated from a fragment of leaf tissue could, within a year, supply 500,000 identical, filariosis-resistant plants producing up to 6 tonnes of oil per hectare per year, that is six to thirty times more than the principal oil-producing plants (sunflower, soya, peanut). This same technique is now being applied to the propagation of new varieties of coconut palms.

Another technique which holds great promise for the future is the *in vitro* production of **haploid plants** (plants whose cells contain a single set of chromosomes). Traditional methods of selection are made more time-consuming and complicated because of the diploid nature of vegetative plants, that is to say, because the cells of which they consist contain a double set of chromosomes, one coming from each parent. As a result, some so-called "recessive" characteristics carried by a chromosome may be masked by a dominant homologous chromosome and its presence may only be revealed, through the operation of Mendelian segregation, after several generations.

This, of course, slows down the work of the person undertaking the selection. The recent emergence of a technique somewhat similar to micro-propagation has made it possible to overcome this difficulty. This technique enables a complete plant to be

obtained either from the male gametes, or reproductive cells (androgenesis), or from the female gametes (gynogenesis). Like the gametes from which they are derived, these plants are haploid. Since they have only one set of chromosomes, their genetic characteristics, whether recessive or dominant, are immediately evident to the person making the selection. Haploid plants are usually infertile, but by treating them with colchicine, which induces a doubling of the chromosomes, a fertile plant is obtained with two sets of identical chromosomes and with phenotypically stable characteristics. Another technique used in gynogenesis is to fertilize the ovule with irradiated pollen.

In China, new varieties of rice obtained by androgenesis are being cultivated on several millions of hectares of land. Laboratory experiments in gynogenesis are also now being undertaken on barley, rice, wheat, maize, sugar-beet and other species.

High hopes are also being placed in **somatic hybridization,** a technique which consists of fusing two cells whose cell walls have previously been removed by enzymatic treatment. Using this technique scientists have succeeded in fusing plant cells not only with other plant cells but also with animal and even human cells. In most cases, however, the chromosomes of one of the fused cells are quickly eliminated and it has only been possible to obtain complete, stable hybrid cells from the fusion of cells from very closely related species. Furthermore, even when stable stock has been obtained, it has proved difficult to regenerate a complete plant from such fused cells. The first success achieved was the regeneration of the pomato, a cross between a potato and a tomato. However, the plant is sterile and remains no more than a laboratory curiosity.

More recently scientists have succeeded in regenerating somatic hybrid cells of several plants of agricultural interest such as rapeseed, chicory and potato. On the other hand, attempts to do the same with sunflower, cereals and legumes have so far failed. Nevertheless, there is hope that present difficulties will soon be overcome, at least in obtaining hybrids of varieties of the same species.

The great advantage of somatic hybridization is that it makes it possible to transfer not only the genetic characteristics borne by the chromosomes of the nucleus, but also those of the specialized parts of the cell contained in the cytoplasm (the "liquid" portion of a cell surrounding the nucleus) such as mitochondria and chloroplasts. These latter are the key to processes and properties of great importance such as photosynthesis, the assimilation of carbon dioxide, male sterility and resistance to herbicides, diseases and drought.

Somatic hybridization has paved the way for the newly emerging discipline of plant genetic engineering which is concerned with the implantation of specific genes, whether of vegetal or other origin, into the genetic make-up of a plant (see article page 13). Using these new techniques the nutritional value of the haricot bean, for example, has been improved by the transfer of a gene from the Brazil nut.

In Europe, Japan and the United States of America, a number of large multinational companies are showing keen interest in these new techniques of plant improvement with a view to competing for the world market. Nevertheless, this branch of biotechnology also offers great opportunities for the developing countries. These new techniques, which they have already acquired or can rapidly master, will enable them to adapt their agricultural production to meet local conditions and requirements.

Nitrogen fixation

Through its World Network of Microbiological Resources Centres (MIRCENs), one of whose priority programmes is devoted to the question of nitrogen fixation, Unesco is contributing actively to another field of biotechnology that is rich in promise (see article page 27).

The *nif* genes, which are coded for the fixation of nitrogen, have now been identified and their structure is on the point of being fully mapped out. Furthermore, these genes have been transmitted to non-nitrogen-fixing organisms such as *Proteus vulgaris, Agrobacterium tumefaciens* and *Escherichia coli*. In principle there is no reason why they should not also be transferred to higher plants and important results in this direction can be expected soon. However, the creation of nitrogen-fixing cereals is a distant prospect still in the realm of science fiction.

With regard to plants other than the legumes, attention is now concentrated on nitrogen fixation by bacteria and fungi which invade their roots either on the root surface or by entering their tissue where they form nitrogen-fixing nodules. These studies have not yet reached the molecular biology or the genetic engineering stage, but they hold out much promise for tropical forestry, sand dune stabilization and the fight against desertification.

Finally, mention should be made of studies being made in the Philippines and Senegal on the use of the water fern *Azolla pinnata* as a biological fertilizer in rice fields (see the *Unesco Courier*, December 1984). In symbiotic association with the blue-green alga *Anabaena* this water fern has the ability to fix atmospheric nitrogen. Ploughed into the soil between harvests, this "green fertilizer" can increase the crop by over 50 per

The Green Revolution

RESEARCH into the selection of new high-yield cereal varieties began after the Second World War. Wheat and rice varieties were selected in Mexico and the Philippines respectively, then during the 1960s the new strains were used in other parts of the world, and it was later established that they had contributed to a significant increase in agricultural yields.

In the mid-1960s, following the introduction of these high-yield varieties into several countries of Asia and Latin America, the expression "Green Revolution" was coined to describe the various efforts made to increase agricultural production in the developing countries by means of these new varieties, especially wheat and rice. The cultivation of these crops required the use of pesticides and irrigation in addition to fertilization and sound agricultural practices. Cross-breeding between these varieties and hardy local breeds made it possible to obtain cultivars that were even better adapted and which gave a better yield. In addition to wheat and rice, this research also concerned millet and sorghum, triticale, maize and several leguminous plant species.

In just over a decade, more than half the surface of corn-growing land and one-third of that of riceland in developing countries had been sown with high-yield cereal varieties. When the latter are irrigated, and receive adequate supplies of fertilizer and weed-killer, the yield is two or three times higher than that of traditional varieties.

The new varieties of wheat were introduced to India in 1966 and Indian wheat production had doubled by 1970-1971, when it reached 23.4 million tonnes. As a result of local efforts to improve varieties and a more widespread use of selected seeds, output reached 33 million tonnes in 1978-1980. From being the world's second largest cereal importer in 1966, India had become self-sufficient by the end of the 1970s. In the Punjab, farm revenues doubled in 1972, six years after the introduction of new cereal varieties.

In some regions of Asia where water resources permitted, the shortening of the growing period of new rice varieties allowed two or three crops to be harvested per year.

The prime beneficiaries of the "Green Revolution" were the wealthier farmers of some developing countries. The countries of Africa south of the Sahara were scarcely affected; only Kenya and Zimbabwe increased the area of land on which new varieties of maize were grown. The wheat and rice varieties were not introduced at the same pace as in Asia where the development of irrigation, adequate fertilizer supplies, and the marketing system of farm produce played an important role in the success of the "Green Revolution." ■

Source: *Quelles biotechnologies pour les pays en développement?* by A. Sasson, Biofutur/Unesco, Paris, 1986

cent and its effect, which lasts for two years, is equivalent to the use of 60 kilograms of nitrogen fertilizer per hectare.

Energy from waste

Biotechnology's contribution in the field of new energy sources is today arousing great interest for two reasons: the foreseeable exhaustion of our supplies of fossil energy (oil and coal), and the world energy crisis which, since 1973, has weighed heavily on the economies of all countries, but particularly on those of the countries of the Third World.

One achievement, which has already been developed on a large scale in a number of countries, is the production of **biogas** from cellulose and animal and human wastes. This is based on the anaerobic digestion of cellulose and nitrogenous organic matter by mixed populations of microbes consisting of bacteria that break down cellulose into organic acids and other bacteria that convert these organic acids into methane.

Experience acquired in India indicates that the manure from ten cows would provide a daily yield of 1.8 cubic metres of biogas, which is the equivalent of 1.3 litres of petrol, enough to cook the food for four people or operate a hundred candlepower lamp for fourteen hours. What is more, the residue constitutes an excellent fertilizer of a quality far superior to the original manure.

A million of these cheap and simple biogas digesters are in service in India and more than seven million are in use in China. Production of biogas on farms can be expected to spread soon to other agricultural areas in which other forms of energy are not available. From the ecological viewpoint, biogas has the great advantage that it can replace firewood, thus contributing to the struggle against deforestation and desertification.

Biogas production is also increasing in the industrialized countries as well as in large towns and heavily populated rural areas in general. The main economic gain here is that the treatment of waste water and the handling of agro-industrial wastes and the animal waste from intensive stock-rearing can be turned to advantage by the production of methane. Already several urban water treatment plants in Europe meet all their energy requirements by the production of biogas.

Green gasoline

The production of **liquid fuels,** in particular ethanol, is another major contribution of biotechnology in the field of new energy sources. A large number of agricultural raw materials can be used for the production of ethanol by fermentation, including the sucrose in sugar-cane, sugar-beet and molasses, the starch from cereals, manioc and potatoes, and the inulin from Jerusalem artichokes.

Brewer's yeast *(Saccharomyces cerevisiae)* and certain anaerobic bacteria, such as *Zymomonas mobilis*, convert the sugars into ethanol with an average yield of 47 per cent, by weight. Several suitable raw materials are available in considerable quantities at a low price. However, from the economic point of view there is one important drawback: the ethanol has an inhibiting effect on the micro-organisms that produce it and the maximum concentration in the reactors cannot exceed 8 to 10 per cent. As a result, the distillation of bio-ethanol and its complete dehydration, which is essential to its use as a fuel, are costly operations constituting about 60 per cent of the cost price.

In Brazil, ethanol fuel is produced from sugar-cane on a large scale. At present production is running at 8.4 million tonnes which in energy terms is equivalent to 5.6 million tonnes of super-grade petrol. In agricultural terms the yield is 4.7 tonnes per hectare of sugar-cane per year.

At present, the cost price of bio-ethanol exceeds that of petrol by $380 per tonne. In Brazil, however, the economic motivation for producing bio-ethanol is to improve the balance of payments by reducing imports of petrol and to provide an outlet for the sugar industry which has been badly hit by the fall in the price of sugar on the world market.

Bio-ethanol is arousing great interest elsewhere for similar reasons. In the United States, "Biohol", an automobile fuel containing 10 per cent ethanol produced from maize, has been on the market for several years. In Western Europe it is planned to produce 3.4 million tonnes of bio-ethanol annually. The aim of this project is to make use of European surpluses of wheat and sugar-beet. There is also an ecological motivation. Added to automobile petrol in a proportion of 5 per cent, ethanol can replace the tetraethyl lead anti-knock additive now used in petrol, but shortly to be banned because of its toxic effects.

Bridging the protein gap

Generally speaking, proteins, or the lack of them, constitute the major nutritional problem facing the developing countries. Statistics published by the Food and Agriculture Organization of the United Nations (FAO), show that average total protein consumption per head of population in the developing countries is only half that of the rich countries. The difference is even more marked with regard to protein of animal origin, average consumption of which in the developing countries is 13 grams per day—a mere 22 per cent of that in rich countries—and this falls to 4 grams per day in the poorest regions of Africa and Asia.

In the developing countries a great variety of agricultural products and wastes lend themselves to the production of **single-cell edible protein.** These include, in particular, ligno-cellulosic matter which is available in large quantities at a low price. According to the United Nations Environment Programme (UNEP), the world crop of cereals produces annually 1,700 million

The cloning of the oil palm

The oil palm (Elaeis guineensis) is cultivated as a source of oil in the humid tropical zones of Africa, the Americas and South-east Asia, where oil palm plantations cover several million hectares. Selection cycles to produce higher-yielding varieties through sexual reproduction were very long, and their results were only perceptible after 15 or 20 years. In the 1970s, attempts were thus made to perfect in vitro propagation of the oil palm using

Photo © IRHO-CIRAD/ORSTOM, Paris

tissue culture, and since 1981 oil palm plantlets have been produced on a semi-industrial scale at the La Mé research station in the Ivory Coast (1) using a cloning technique developed by British and French researchers in the 1970s. Photos show some of the stages in the cloning process. Fragments of very young leaves are carefully removed from the tip of a tree (2) and placed in a nutrient medium where calluses develop (3). After going through a second and then a third culture medium the calluses evolve into "embryoids" (4) comparable to the embryos obtained by sexual reproduction. They multiply spontaneously, and this multiplication is fostered in a fourth culture medium. A fifth culture makes it possible for the embryos to develop into young leaved plantlets (5). The shoots are transferred to a sixth medium in which roots are induced (6), while in a seventh medium entire young plants are obtained for planting in soil (7). It takes about 3 months to obtain a 12 cm shoot from an embryoid.

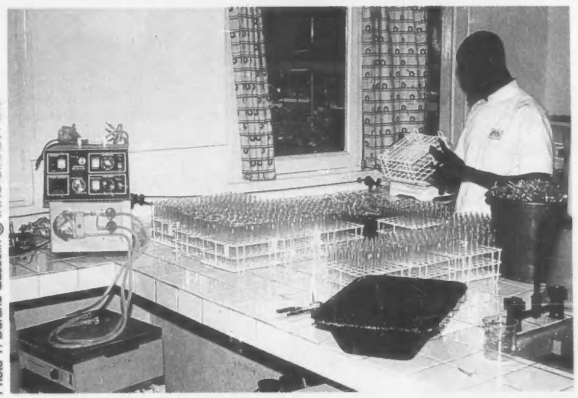


Photo T. Durand-Gasselin © IRHO-CIRAD, Paris

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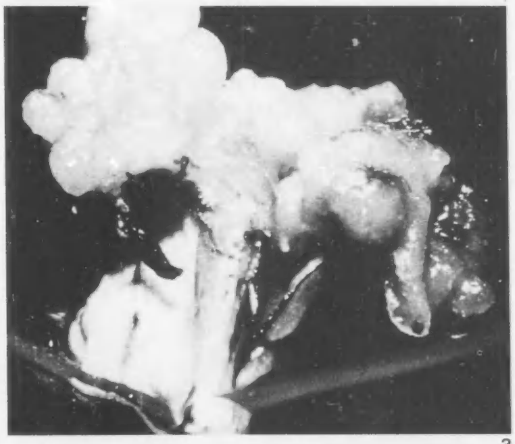

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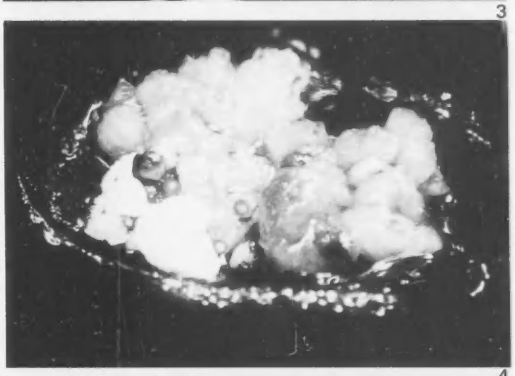

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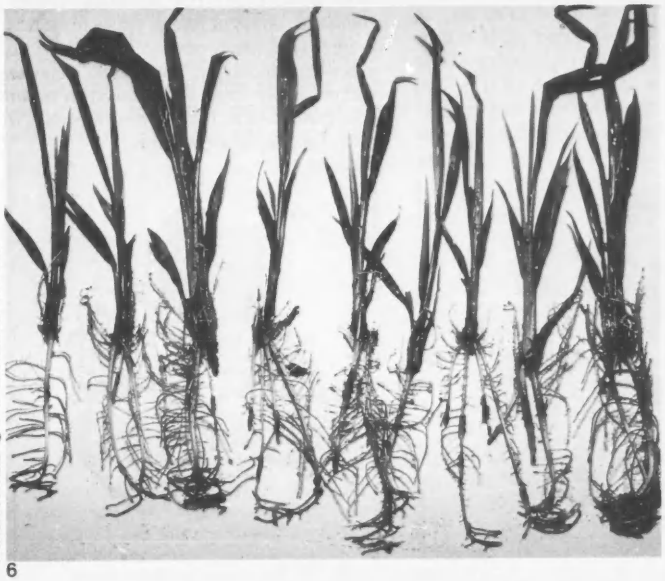

Photos C. Panetier © IRHO-CIRAD/ORSTOM, Paris

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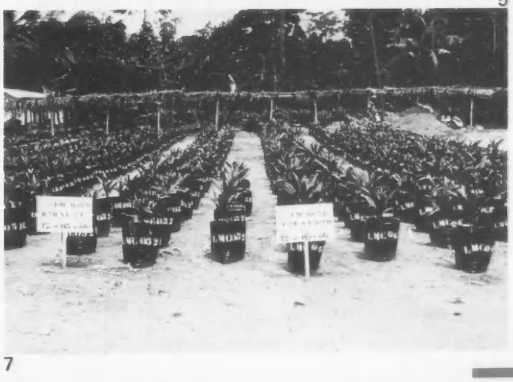

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1

Powerful protoplasts

Techniques for the cloning of plants are now so refined that a single cell removed from the body of a plant can be cultured in the laboratory and then induced to regenerate a complete individual plant. Drawings at left and below are a schematic representation of the cloning process used by Prof. James F. Shepard and his colleagues at Kansas State University to regenerate a complete potato plant from protoplasts (living cells stripped of their outer wall) prepared from leaf cells. Small terminal leaves are first removed from a young potato plant (1). The leaves are placed in a solution containing a combination of enzymes capable of dissolving the cell wall to produce protoplasts (2). The solution also causes the protoplasts to withdraw from the cell wall and to become spherical, thereby protecting the protoplasm during the disintegration of the walls (3). The protoplasts are next grown in a culture medium (4) where they divide and begin to synthesize new cell walls (5). After 2 weeks of culture in these conditions, each protoplast gives rise to a clump of undifferentiated cells or microcalluses (6). These microcalluses develop into full-size calluses in another culture medium (7) and their cells begin to differentiate, forming a primordial shoot (8). The shoot develops into a small plant with roots in a third culture medium and is then planted in soil (9). Under appropriate conditions protoplasts from 2 different plants can be fused to form a cell possessing genes of plants which cannot be crossed using classic methods. The fused protoplasts of some species can be grown into plants in a process known as somatic hybridization.

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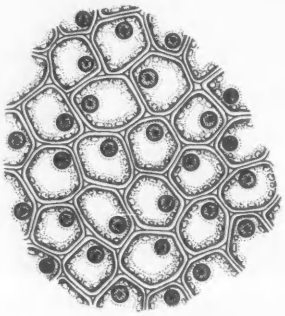

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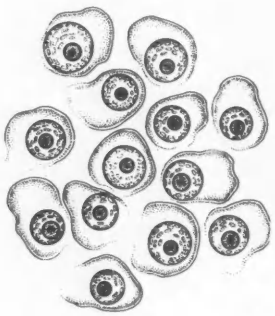

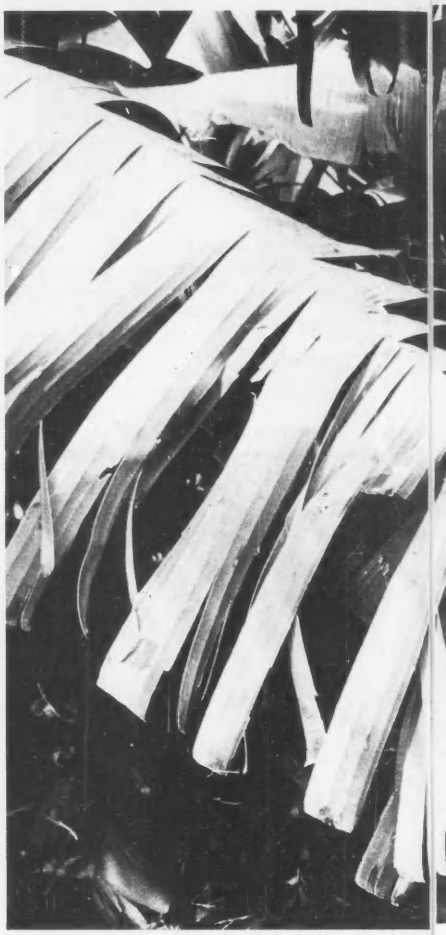

tonnes of straw, to which can be added some 127 million tonnes of bagasse from sugar-cane and pulp from sugar-beet. At present, the main obstacle to their use for the production of proteins is the lack of sufficiently active microbial strains for this specific purpose. Recently achieved laboratory results suggest that this problem will soon be overcome.

Cuba is, at present, the only developing country producing single-cell edible protein from agricultural raw material. Eighty thousand tonnes of forage yeasts for use as animal feed are produced annually from sugar-cane molasses. The Cuban example will probably soon be followed in other countries, such as India, where molasses is also available at a low price.

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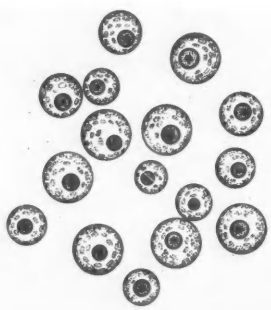

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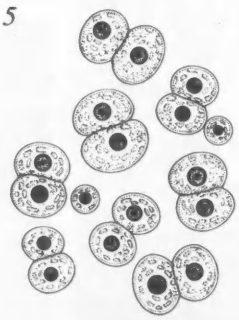

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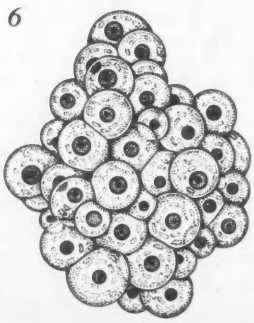

Photo Yves Jeanmougin © Rapho, Paris

Protein enrichment by fermentation is a branch of biotechnology that could help some developing countries increase their protein resources. Microbial fermentation of such crops as manioc, which contain much starch and relatively little protein, yields a product with a substantially higher protein content. The banana is a fruit to which this process could be applied, and several banana-producing countries are investigating the possibility of using in this way the high proportion of fruit rejected for export and usually wasted. Above, harvesting bananas in Martinique.

For a long time now Western Europe has been producing single-cell edible protein from various agro-industrial wastes such as lacto-serum (80,000 tonnes per year) and the sulphite liquors used in paper-making (25,000 tonnes per year). As with biogas, the main economic incentive for this production is the elimination of the cost of handling potentially polluting wastes. It is to be expected that the same will happen soon in the developing countries where increasing industrialization is making protection of the environment an ever more urgent necessity.

One of microbiology's most promising contributions to the problem of edible proteins is their production on an industrial scale from oil, methanol and natural gas. Born in Europe some thirty years ago, this branch of biotechnology has developed to the point where there are factories with a production capacity of 100,000 tonnes per year.

The oil treatment processes make use of yeast micro-organisms *(Candida lipolytica* and *Candida tropicalis)* which are derived from diesel oil or from paraffin, previously extracted from crude oil, and having a yield of 100 per cent by weight. In the case of methanol, chemically derived from natural gas, the biomass produced is that of bacteria such as *Methylophilus methylotropha* whose yield on this substrate is of the order of 50 per cent, by weight. The methanol treatment processes make use of specific methane-eating bacteria *(Pseudomonas meth-*

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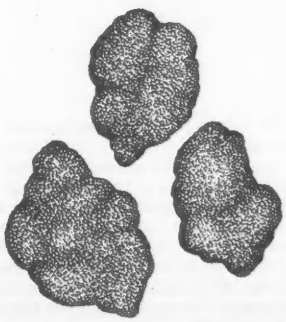

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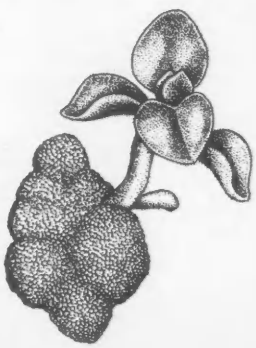

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After J.F. Shepard in *Scientific American*, New York, 1982

ylotropha or *Methylococcus capsulatus)* in conjunction with other species whose task is to prevent the inhibition of the bacteria by intermediate accumulation of methanol.

Very large-scale experiments with the products thus obtained from oil and methanol have demonstrated conclusively their high nutritional value and complete innocuousness. Up till now, these edible proteins have been marketed exclusively as animal feed, but preliminary studies have shown that there is nothing to prevent their being used directly as food for humans.

Following the first oil crisis of 1973, production of single-cell edible protein from oil and from methanol slowed down in Western Europe due to the increased cost of the raw material. In Eastern Europe and in the USSR, however, it has developed considerably and now amounts to some 3 million tonnes per year.

This branch of biotechnology is of obvious interest to those developing countries that are producers of oil and natural gas, since these raw materials are available to them in large quantities at prices well below the world market price. The Organization of Arab Petroleum Exporting Countries, for example, proposes as a first step to produce 100,000 tonnes of single-cell edible protein a year, either from oil or from methanol, and it estimates the potential market in the Middle East and in the Maghreb at more than a million tonnes. It should be pointed out that this amount of protein could be obtained from 0.1 per cent of their total oil production.

Protein enrichment of foodstuffs by fermentation is another promising prospect. Application of modern biotechnological methods to this practice, which is traditional in Africa and the Far East, seems set fair to provide the developing countries with a substantial increase in their protein resources for human and animal consumption.

The end product of this fermentation process is a directly consumable mixture of protein-rich microbial biomass and residual agricultural raw material whose nutritional value is thus enriched. This relatively simple technology has the advantage that it can be used both on a large industrial scale and in small, inexpensive production units located in rural communities. This means that high-quality edible protein can be produced from a wide range of agricultural raw materials that are too costly or available locally only in quantities too small for use with standard single-cell edible protein production methods.

In all the tropical regions, manioc (also known as cassava) is the chief agricultural raw material potentially available for protein enrichment. Cultivated throughout Africa, in Asia and Latin America, the world production of manioc is of the order of 100 million tonnes. Very rich in starch, but containing practically no protein, manioc is used above all as a supplementary energy food. Furthermore, although under good conditions it can yield 50 tonnes per hectare and over, it is normally only cultivated on small patches of land using rudimentary methods with low productivity.

At present, the only country in which manioc cultivation has been developed rationally is Thailand, which exports 7.5 million tonnes of manioc root to the European Community each year.

Starting with dried manioc with an initial content of 90 per cent starch and less than 1 per cent protein, fermentation with an amylolytic mould *(Aspergillus hennebergii)* yields a product containing 20 per cent well-balanced proteins and 20 to 25 per cent residual sugars. In this way manioc can provide nearly 2 tonnes of protein per hectare, that is, three times more than can be obtained from the cultivation of soya or other leguminous plants.

The banana too is a raw material with a bright future. In the collection centres of exporting countries, 20 to 30 per cent of the fruit gathered is rejected. This rejected fruit, whose protein content at 1.1 per cent is too low for it to be used as animal feed, is a complete write-off. For a number of Central American countries which export several million tonnes of bananas annually, the prospect of recuperating wastage on this scale by use of the fermentation process is clearly of the greatest interest and this possibility is being actively investigated in Mexico, Guatemala and the West Indies.

Finally, the third major contribution that biotechnology has to offer to the solution of the world problem of edible protein is the **industrial production of amino acids** as a complement to plant proteins. Many such proteins, in fact, are only of limited nutritional value because of their lack of certain essential amino acids which man and other mono-gastric animals (including pigs, young ruminants and poultry) are unable to synthesize and therefore must find in their food. This is the case in particular of lysine, which is the amino acid in which cereals are most notably deficient and lack of which is the main cause of malnutrition in the Third World. Almost all the amino acids used as a complement to plant proteins are obtained by fermentation using hyper-productive bacterial strains selected genetically.

Apart from methionine, which is basically intended for use in animal feed, lysine is the only amino acid produced in large quantities (40,000 tonnes per year). It has been estimated that the world deficit in lysine, most marked in Africa and the Far East, is 136,000 tonnes for human food and three times that figure for animal foodstuffs. As things stand at present, the cost price of lysine is still too high to ensure satisfaction of Third World needs and to compete with soya in animal feed. The situation is the same for other amino acids, in particular threonine and tryptophan, which, after lysine, are the chief elements lacking in plant proteins. However, it is reasonable to assume that, thanks to genetic engineering, substantial progress will soon be made. ■

JACQUES C. SENEZ, *French biologist and university teacher, is a former Secretary-General of the Unesco-sponsored International Cell Research Organization (ICRO) and a consultant member of the Protein Advisory Group of the United Nations. A past Secretary-General of the International Union of Microbiological Societies (IUMS), he is the author of a number of studies on microbiology and bacterial biochemistry. In the late 1960s he initiated the production of Single Cell Protein (SCP) from petroleum.*

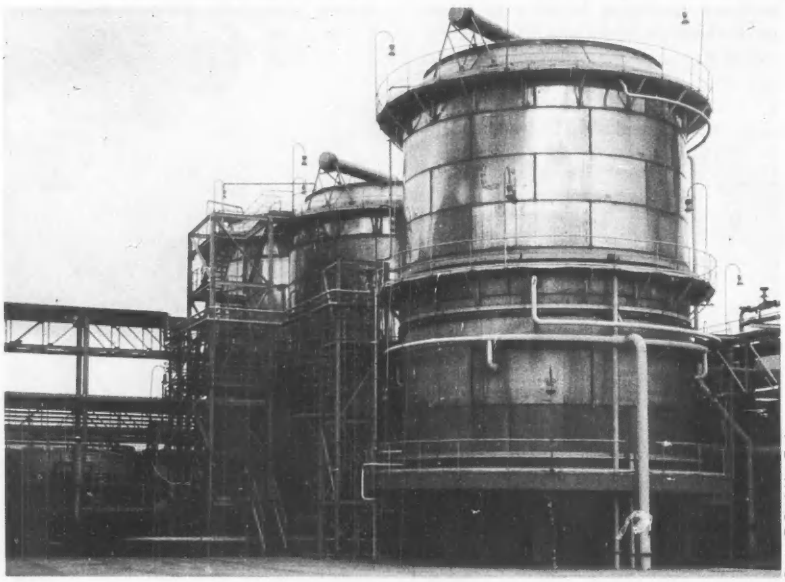
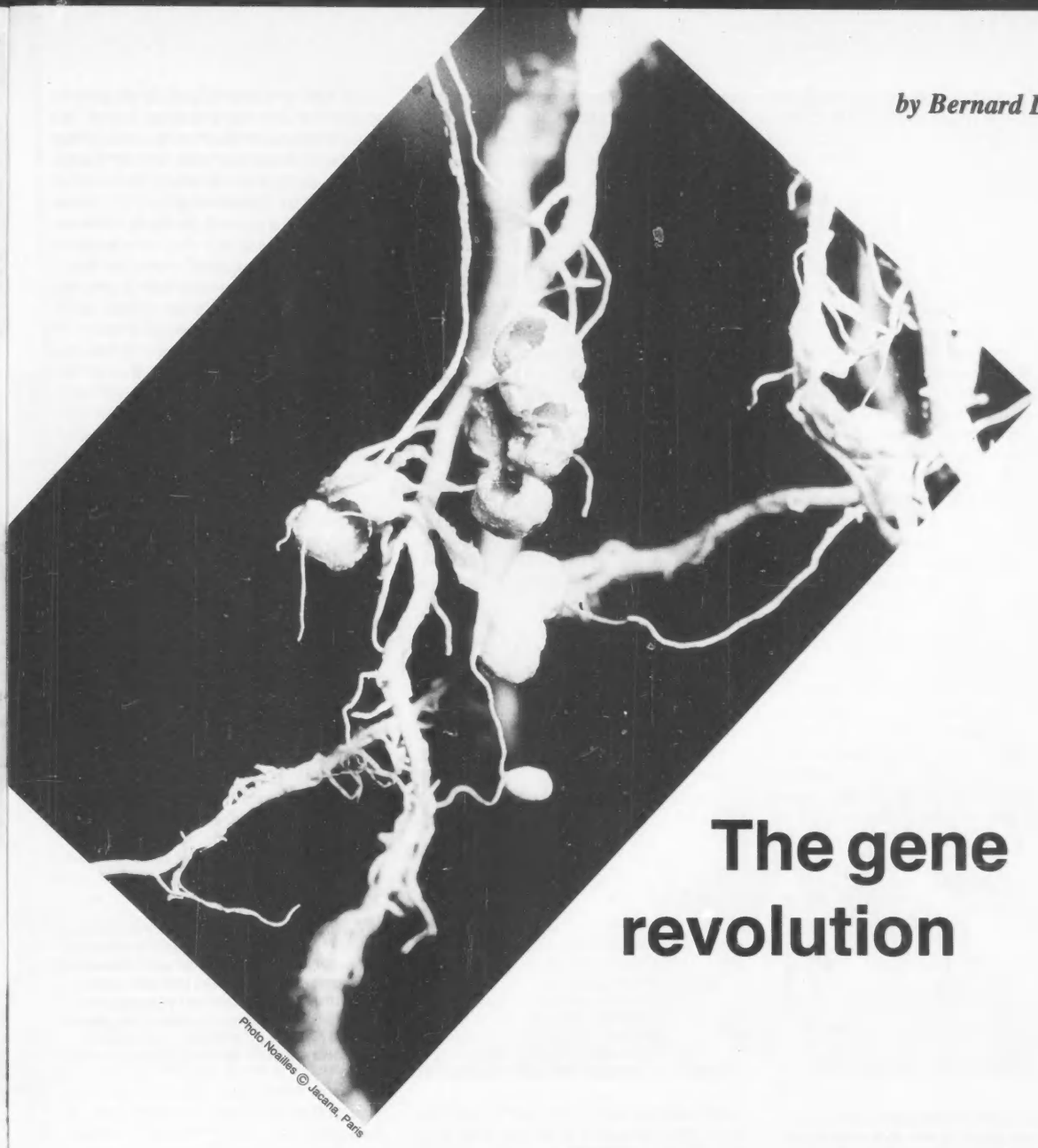


Photo © Speichim, Paris

Many developing countries are engaged in programmes to harness the techniques of biotechnology for national development. Left, fermenters of a Cuban factory producing single-cell edible protein from molasses. The installation produces some 40 tonnes of protein a day for use as animal feed.

by Bernard Dixon



The gene revolution

Photo above shows the distinctive knot-like growths or nodules which form on the roots of legumes (plants of the pea family) when they are infected by certain bacteria. These bacteria, known as rhizobia, take nitrogen from the air and change it into forms the plants can use. One important aim of research in biotechnology is to extend this process of nitrogen fixation to other crops by incorporating nitrogen-fixing genes into their genetic heritage. The goal is proving difficult to attain.

GIVEN the mixture of benefits and problems spawned by the first Green Revolution two decades ago (see box page 7), it is not surprising that both optimism and apprehension surround the application of genetic engineering now to agriculture tomorrow. Mixed reactions *are* appropriate, because those developments—focused upon so-called recombinant DNA—are destined to have even more far-reaching effects than the techniques deployed in the first revolution. Today's new wizardry could undoubtedly transform agriculture throughout the world. At the same time, its precision in modifying living cells offers a stern challenge to our prudence and wisdom.

At the centre of the stage is deoxyribonucleic acid (DNA), the material which carries in coded form the hereditary instructions responsible for the behaviour of cells and the plants, animals or microbes of which they are part. The astronomically long DNA molecule can be subdivided into regions—genes—which determine particular characteristics. Recombinant DNA is the name given to the product when a piece of DNA from one organism is combined artificially with that from another.

Genetic manipulation of this sort is the basis for the boom that has occurred during the past decade in biotechnology. Such activities were, of course, possible previously. Some, like the art of fermenting sugar to make alcoholic drinks, are almost as ancient as Man himself. Others, including the first mass production of antibiotics, were developed earlier this century. But all of these processes were based on organisms as they occur in nature—albeit with other, equally natural, methods being used to select high-yielding strains.

The arrival of recombinant DNA, however, has altered the rules profoundly. It

has already greatly enhanced our specificity and power in tailoring living organisms for beneficial purposes. In future, it will extend our range of options much further.

The breakthroughs which have led to this historic watershed in the fabrication of novel plants and microbes happened during the early 1970s. The key discoveries were made by molecular biologists who learned how to splice into bacteria genes which they had taken from other bacteria, and even from totally unrelated animal or plant cells. They first found out how to locate the particular gene they wanted among the countless numbers on the DNA of one organism. Then they used natural catalysts called enzymes to cut out that gene and "stitch" it into a *vector*. This is usually a virus or a plasmid (a piece of DNA that replicates independently from the nucleus, the main repository of DNA). The vector became a vehicle for ferrying the selected DNA fragment into the recipient. Once inside its new host, the foreign gene divided as the cell divided—leading to a clone of cells, each containing exact copies of that gene.

Because the enzymes used for genetic engineering are highly specific, genes can be excised from one organism and placed in another with extraordinary precision. Such manipulations contrast sharply with the much less predictable gene transfers that occur in nature. They also make it possible to splice genes that would be unlikely to come together naturally. By mobilizing pieces of DNA in this way, genetic engineers are beginning to create pedigree microbes for a wide range of new purposes in agriculture, medicine and industry.

Although genetic manipulation is taking longer to perfect in plants, several techniques are now emerging. The most useful so far is based on *Agrobacterium tumefaciens*, a bacterium that causes crown galls on many flowering plants. It contains a tumour-inducing (Ti) plasmid which is responsible for triggering the disorderly growth that appears as ugly galls. Genetic engineers have learned how to delete the Ti plasmid's tumour-inducing genes and use it as a vector with which to carry new genes into plants.

A serious drawback so far is that while *A. tumefaciens* infects potatoes, tomatoes, and many forest trees, it does not normally attack the monocotyledons such as cereals, which are prime targets for genetic improvement. Progress is being made, however, and recent research indicates that rice in particular can be manipulated using the Ti plasmid. Alternative vectors and other methods of transferring genes are also being developed. One exciting possibility is to use an electric current to promote the incorporation of foreign DNA. This works with maize cells, though scientists still have to persuade the cells to develop into whole plants.

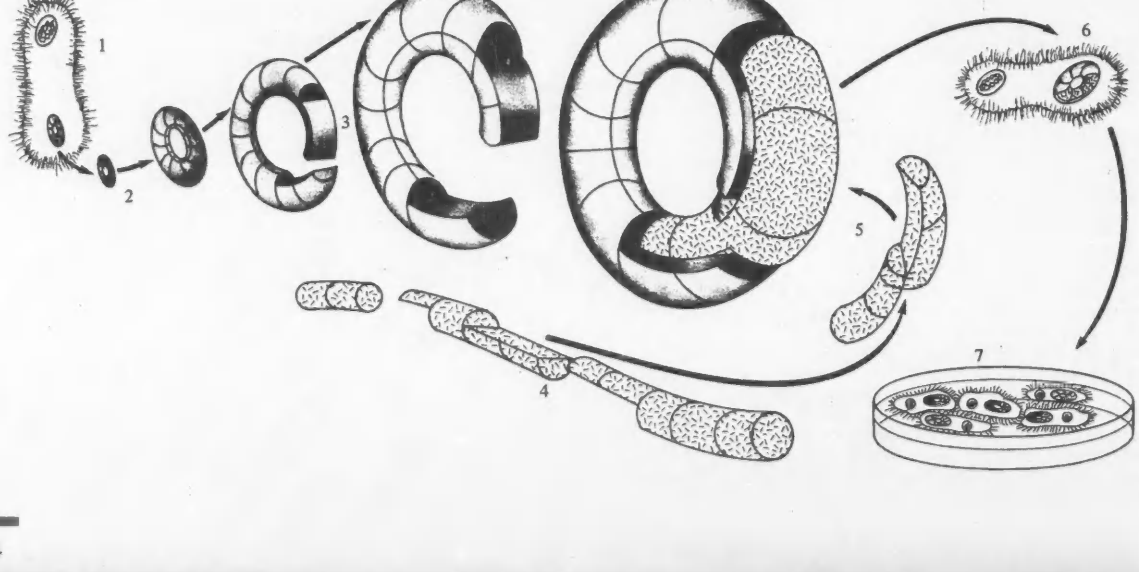
One gene that has been transferred into tobacco by *A. tumefaciens* comes from bacteria and gives the plants the capacity to produce a toxin that is lethal to insects. The inbuilt insecticide makes the plants resistant to insect attack and does not, of course, have to be applied repeatedly. Some plants can mobilize defences against virus infection through a process analogous to immunization in animals, and this suggests another route for genetic alteration. Incorporation of one virus gene into tobacco has helped to protect this plant against subsequent inoculation with the entire virus.

Another development concerns weeds — a major limitation on crop husbandry in most countries. Although weeds can be combatted using selective herbicides, these often impair the growth of the crop too. It is now possible, however, to introduce resistance genes into tobacco and petunia. One such manipulation results in the synthesis of enzymes in the plant that are no longer sensitive to the inhibitory action of the herbicide glyphosate. Commercial companies now plan to market a package containing both herbicide and resistance seed.

Some 70 per cent of the world's intake of dietary protein consists of cereal grains and seeds of legumes. On their own, neither cereals nor legumes can provide a balanced diet for human consumption, because the "storage proteins" they each contain are deficient in one or more amino acids. Now, added to analyses of the proteins in both cereals and legumes, we have precise infor-

How to recombine DNA

Drawing shows how a micro-organism (in this case a bacterium) is manipulated to make it synthesize a desired substance. (1) A bacterium contains a plasmid, which is a circular piece of DNA. This plasmid is isolated (2) and, with the help of a restriction enzyme, opened in a precise spot (3). Meanwhile, with the help of other restriction enzymes, the gene for synthesis of the desired substance is isolated from the DNA of another organism (4). Still using enzymes, this gene is grafted onto the previously opened plasmid (5). The plasmid is re-introduced into a bacterium (6). The manipulated bacteria are put into a culture, where they synthesize the desired substance. (7)



Source FAO-Ceres, Nov-Dec 1984

mation about the DNA sequences coding for them. This knowledge may well lead to methods of altering those sequences or introducing new genes that code for a more balanced spectrum of amino acids.

The world's energy and food supplies rest upon the ability of green plants to convert atmospheric carbon dioxide into carbohydrates, fats and proteins, using light from the sun. Unfortunately, the mechanism by which they consume carbon dioxide is inefficient in those plants, such as wheat, barley and potatoes, that are cultivated in temperate climes. Oxygen in the atmosphere interferes with the first enzyme involved in the assimilation of carbon dioxide. Considerable efforts are now being made to alter the DNA sequence of the gene coding for this enzyme, to prevent the deleterious action of oxygen. Other researchers are trying to introduce into temperate zone plants certain genes taken from maize, which has a more efficient mechanism of carbon dioxide uptake. In nature this mechanism appears to operate only at higher temperatures, but there are hopes of "switching it on" in cooler areas.

Another atmospheric gas is the subject of parallel efforts to make plants more efficient. Nitrogen constitutes 80 per cent of the air, yet plants cannot use the gas directly. Hence the heavy dependence of modern intensive agriculture on fertilizers—nitrate, ammonia or urea—synthesized by the chemical industry. Natural nitrogen fixation depends in part on rhizobia, bacteria that live symbiotically with legumes such as peas, beans and clover. The bacteria grow on sugars provided by the plant, and are maintained in characteristic nodules on the plant. There they convert nitrogen directly into ammonia, leading in turn to the synthesis of plant proteins.

Molecular biologists have now isolated and characterized several of the genes required for nitrogen fixation. They have found, however, that many more bacterial *and* plant genes are involved than they first imagined. This makes the manipulation of those genes correspondingly more difficult. So it will be some years before we can enjoy the cost and energy savings that should accrue by providing crops such as wheat and maize with the ability to fix their own nitrogen.

Drought and high temperatures are unwelcome to all plants, despite being better tolerated by varieties that have evolved in such environments. Desiccated soils also often contain high levels of salts and metallic elements, which are toxic to plant growth. Genetic engineers would dearly like to fabricate plants resistant to such stresses, but success is unlikely in the near future. Before being able to identify the relevant DNA sequences for transfer between plants, they require a far better understanding of the many ways in which plants respond to their environment. An additional problem may be the involvement of several different genes, as with nitrogen fixation. Drought resistance which depends on a reduced area of leaf surface, for example, may be caused by the interaction of multiple genes.

Microbes that contribute to healthy plant growth are also on the drawing board for genetic engineering. One possibility being examined is the production and deliberate release of rhizobia that fix nitrogen more efficiently than natural strains. Other bacteria capable of forming nitrogen-fixing partnerships with wheat and maize are also being considered. A third type of prospect follows the discovery by researchers at the University of California, Berkeley, that frost damage to strawberries is triggered by bacteria which act as nucleii for the formation of ice crystals on leaves. The cause is a particular bacterial protein, the gene for which the California biologists have learned to delete. They believe they can prevent the extremely costly frost damage by spraying crops with this "ice minus" strain, which will outgrow the natural flora.

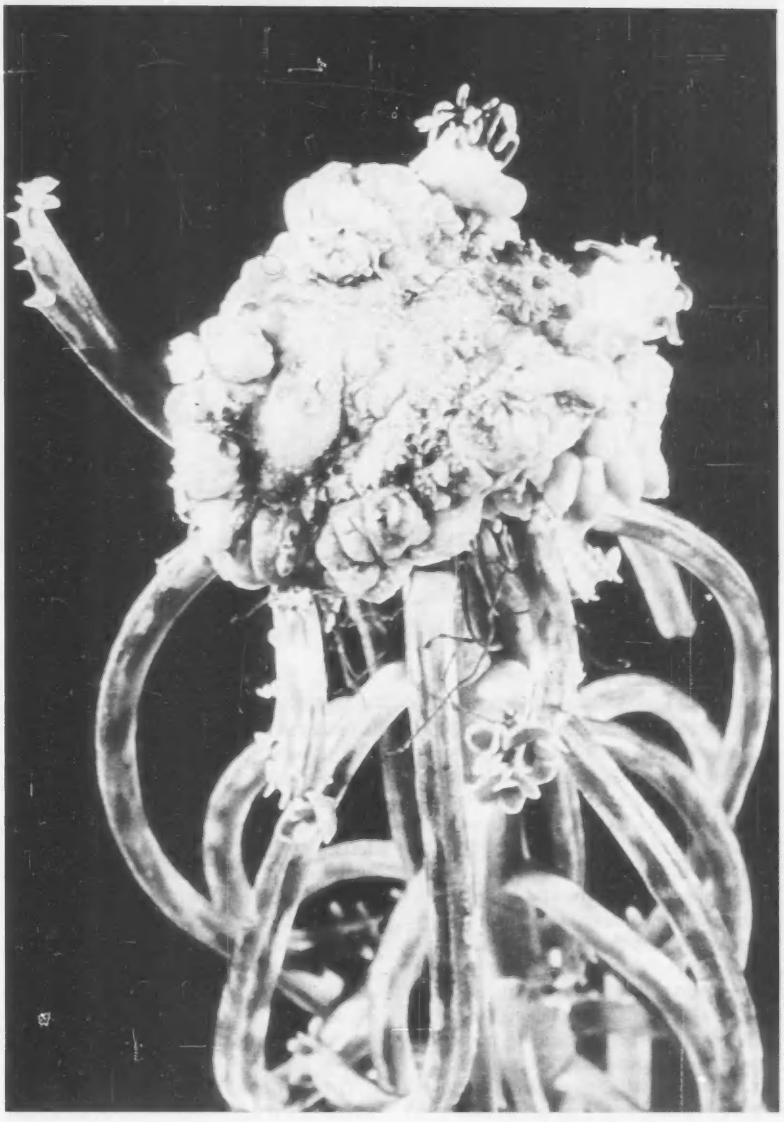


Photo D. Tepfer © INRA, Paris

A key area in biotechnology research is concerned with the development of techniques for isolating genes of one plant and introducing them into another as a means of endowing the host plant with new characteristics such as higher protein content or resistance to pests. One promising technique for transferring genes uses plasmids (small pieces of genetic material) from a bacterium which causes tumour growths when it infects certain plants, above. It is possible to delete the plasmid's tumour-inducing genes and use the plasmid to ferry new "useful" genes into plants. Genes of a bean protein have been transferred to the sunflower using this method.

Source FAO-Ceres, Nov-Dec 1984

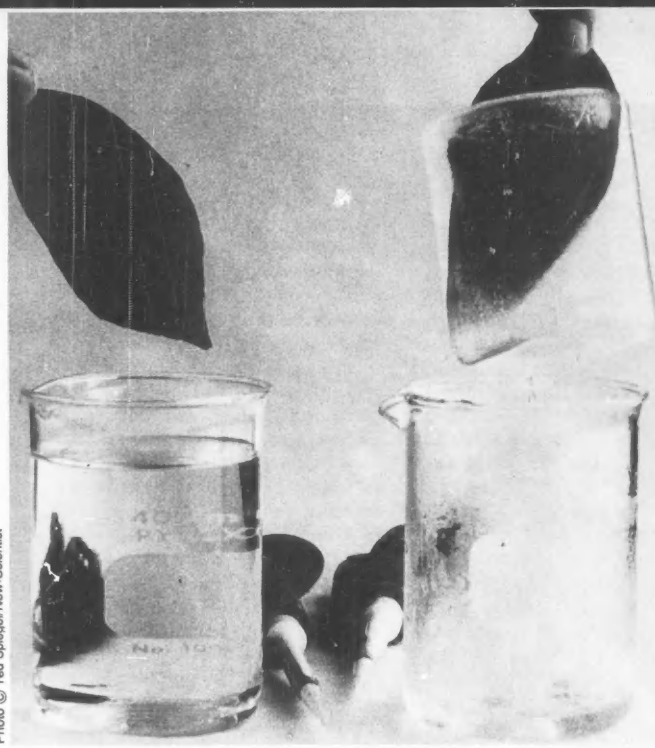


Photo © Ted Spiegel/New Scientist

The use of genetic engineering in food production offers many potential advantages. At the same time, questions have been raised about the possible risks involved in releasing genetically modified living organisms into the environment. One case which led to widespread debate and concern in the United States arose from the development and use of genetically modified microbes known as ice-minus bacteria to protect strawberries from frost damage. In photo, the leaf at left has been treated with ice-minus bacteria. The leaf at right froze when dipped into supercooled water.

Genetic engineering holds considerable promise too in the improvement of "biological insecticides", microbes that attack pests and have enormous ecological advantages over their chemical counterparts. *Bacillus thuringiensis,* for example, has been used for many years to combat nuisance species, but it and similar bacteria and viruses may well be made more powerful by recombinant DNA. One possibility is illustrated by the pine moth, which damages lodgepole pine trees in northern Britain. In other parts of the country, the moth is controlled naturally by a baculovirus that infects the caterpillars. There are now plans to make the virus more efficient at killing caterpillars and to release it in the pine plantations. The first experiments are being carried out with a virus that has been altered only by having a "marker" introduced into a non-coding region of its DNA. This will allow researchers to follow the virus's distribution and survival after spraying. If all goes well, the virus may be given a gene allowing it to synthesize an insect-killing toxin. The potential for this technique in other countries, against other destructive insects, is clear.

The safety of laboratory and industrial activities using engineered organisms is based on the idea of containment. Facilities are graded according to the degree of risk. New questions arise, however, when microbes and plants are to be introduced into the environment. There is concern, for example, that weeds may be created accidentally and be inordinately difficult to eradicate. If such a plant were drought-resistant, herbicide-resistant, *and* frost-tolerant, it might spread quickly over large areas of agricultural land and be very difficult to eradicate. As illustrated by the Kudzu plant in Asia and the water hyacinth in America, even natural weeds can cause considerable havoc.

The prospect of genetically engineered crops themselves becoming weeds is remote, however, because crop varieties cannot compete well with other plants when left unattended. The inherent difficulties of mobilizing plant genes also make it unlikely that unwelcome varieties will be produced accidentally. And there is always the possibility of destroying by fire or other means an engineered plant, released initially in a defined area, that *did* create problems. Nonetheless, field trials with novel plants, particularly crops able to cross-fertilize with weeds, need to be very carefully monitored.

Greater caution still is required with engineered microbes, which would be broadcast in astronomical numbers and be impossible to trace in their entirety should anything go awry. But it is reassuring that no health, environmental or other dangers have been caused by recombinant organisms since they were first fabricated over a decade ago. Moreover, biologists now agree that there is no significant difference between a microbe that has received a new piece of DNA through artificial manipulation and one that has acquired the same DNA fragment through natural mechanisms of gene transfer. Most experts argue that recombinant DNA manoeuvres are intrinsically safer, because they can be vastly more precise and selective. Certain laboratory manipulations are ruled out anyway by *a priori* predictions that they would generate hazardous recombinants.

Many researchers believe that tests with recombinants should always be restricted to closed environments such as greenhouses. But these "microcosms" can never simulate the richness of the natural biosphere. So they can never provide conclusive evidence about an organism's potential safety or performance in nature. The scientific consensus is now for a gradual approach, *a priori* evidence about a released organism's likely behaviour being used as the basis for successively larger trials during which experience and confidence are gathered about how it actually does behave.

There is one other argument against the much-publicized view in the USA that organisms carrying recombinant DNA should *never* be released for purposes such as pest control. One third of the world's crops are now lost through infection and pestilence. It would be foolhardy not to make use of an ecologically acceptable technique capable of achieving even a modest reduction in that toll. ■

BERNARD DIXON, *British science writer and consultant, is European editor of* The Scientist *magazine and was formerly (1969-1979) editor of the British scientific journal* The New Scientist. *Notable among his published works are* Magnificent Microbes *(1976),* Invisible Allies *(1976) and (with G. Holister)* Ideas of Science, Man and Medicine *(1986).*

Photo © Hitachi Ltd., Tokyo

Tomatomation

Japan's high-tech food factories

by Koichibara Hiroshi

Light, temperature and humidity are computer controlled in this vegetable factory in a Tokyo suburb. High electricity consumption is a drawback.

THE harnessing of high technology to vegetable farming may be about to trigger a new agricultural revolution in Japan, where some large manufacturers are already offering fully automatic "factories" in which vegetables are grown in a computer-controlled artificial environment. In their use of automation and high technology these facilities resemble automobile or electronics plants, but instead of automobiles or video tape recorders their mass production lines produce fresh vegetables, regardless of season or climate.

Strictly speaking, today's factory farming technology is based not on biotechnology but on applying industrial production management techniques to conventional agricultural engineering. The aim is to use artificially controlled environments to grow plants rapidly and efficiently rather than improve the adaptation of plants to natural conditions. Such ideas have already been applied to poultry farming, egg production systems, and even the production of *foie gras*. Factory farms may thus make a big impact on conventional agriculture since they provide planned cultivation regardless of weather, season, climate or soil.

The essential element in this new development is hydroponics, the cultivation of plants in nutritive solutions. Factory farms are air-conditioned, and high-pressure sodium lamps provide twenty-four-hour-a-day illumination. The density of carbon dioxide, oxygen, temperature and humidity are controlled by a computer to maintain an optimum growing environment.

The hardware used in this process is not new. It is readily available from manufacturers of electrical consumer goods, and this may be the reason why Japanese electrical conglomerates are active in this field. Companies in Denmark, the United States and Austria are also experimenting with vegetable factories but for the moment the Japanese seem to be leading the field.

In 1985, a "supertomato" plant was displayed in the Japanese government-sponsored pavilion at an international exhibition held in Japan, Tsukuba Expo. '85 (see the *Unesco Courier*, March 1985). This was a major success for a hydroponic culture system developed after many years of research by a Japanese agronomist, Nozawa Shigeo. The growth of the plant was accelerated in a nutritive solution which replaced soil and in an artificially controlled environment. As a result the plant produced more than 13,000 tomatoes during the six months of the Expo.

Daiei, Japan's biggest supermarket chain, has installed a factory farm next to its store in the Tokyo suburb of Fanabashi. This experimental facility, constructed in co-operation with Hitachi Ltd. to grow lettuce for sale in the adjoining supermarket, may be the world's first commercial factory farm using full automatic hydroponic culture technology. The system produces some 130 heads of lettuce and other green vegetables per day (some 47,000 per year) on a floor space of no more than 66 square metres. Grown from seed, the lettuce is big enough for harvesting in only five weeks, 3.5 times faster than plants cultivated using conventional methods.

In this futuristic factory, the sun is replaced by artificial twenty-four-hour lighting, soil with nutritive solution and

farmers with a micro-computer. The crop is tasty and free from pesticides and herbicides, and is in great demand, regardless of the price tag, which is double that of conventionally grown lettuce.

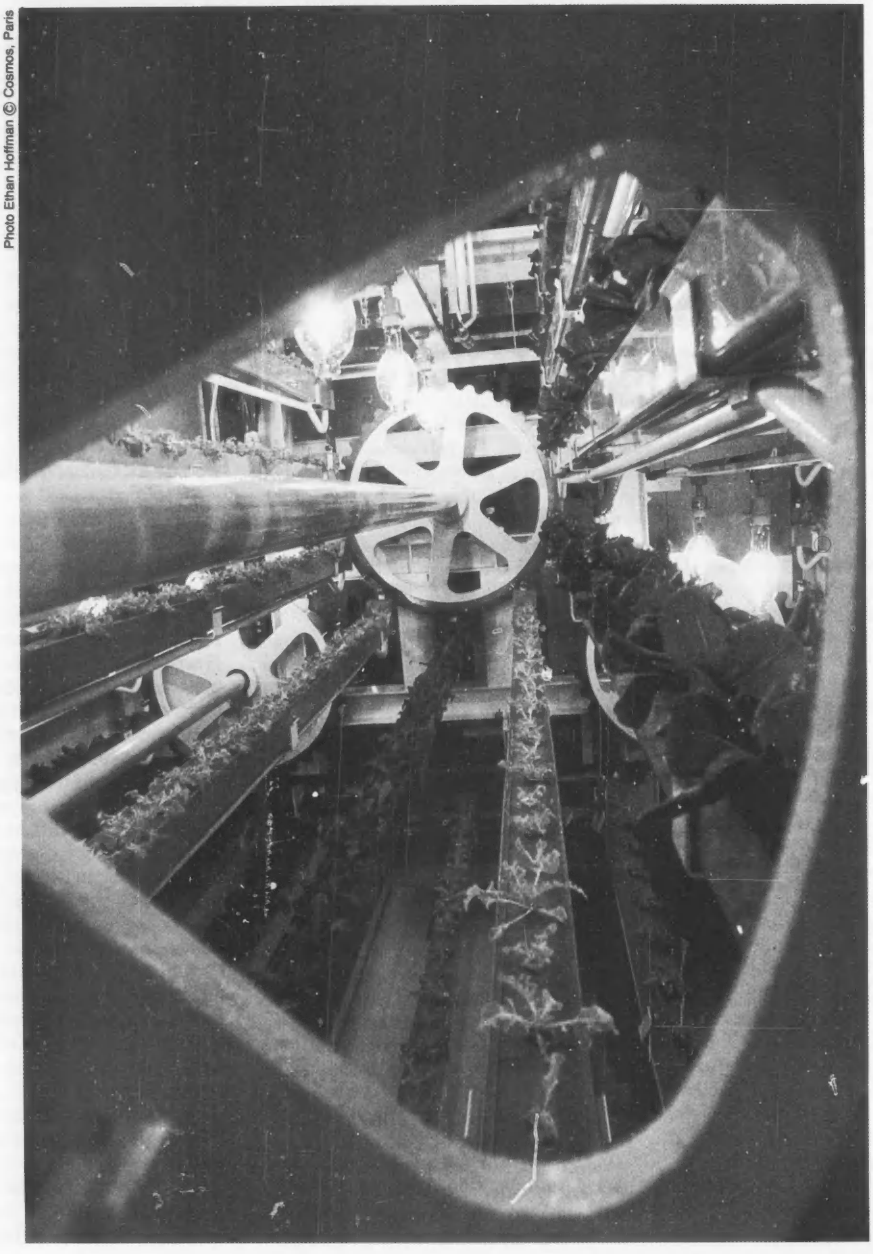
In Mitsubishi Electric's Amagasaki laboratory, a prototype food factory assembly line succeeded in growing lettuce seedlings from 2 grams to 130 grams in 15 days—6 times faster than the natural growth rate. With specially developed fluorescent lamps, the photosynthetic ratio is said to be better than that of the sun. Sprouts cloned from the tissues of mature plants start at one end of a conveyor and move along at the rate of 20 centimetres a day.

In March 1986 Japanese National Railways (JNR) built two experimental vegetable factories, each with a size of 50 square metres and a construction cost of $60,000. Since May, each factory has been producing 120 heads of lettuce a day. Experiments are being carried out on the cultivation of other vegetables such as tomatoes, cabbage, asparagus, melon and green peppers. In the case of JNR, electric power supplied by its own power plants can be efficiently used at night when demand is low, and open spaces beneath the overhead railway or abandoned tunnels can be utilized as sites.

Artificial lighting and computers are not essential elements in factory farming. Hydroponic food factories can be installed in developing countries where food factories may be most needed. Matsushita Electric has, for example, installed a vegetable factory with minimal automation in the Maldives. The system, which has a plastic

Trays of growing lettuce were rotated up and down on chain conveyors in this vegetable factory installation shown in the Japanese Government pavilion at Expo '85, an international exhibition held at Tsukuba (Japan) in 1985. The lettuce were grown in liquid nutrients, using the technique known as hydroponics. The 24-hour-a-day lighting, carbon-dioxide-rich atmosphere and constant temperature helped the lettuce to reach maturity in 20 days, 4 to 5 times faster than normal. The moving conveyor belts ensured that every plant was exposed to the same amount of heat and light.

Photo Ethan Hoffman © Cosmos, Paris



Photos © AFP, Paris

roof that keeps out harmful sunlight rays, produces 50 tonnes of vegetables a year, using about one-fifth of the water needed by field-grown plants.

Vegetable factories can offer various advantages: planned production, quality control, low labour costs, clean products. They use space efficiently and provide stable production regardless of climatic and seasonal variations. However, high electricity costs are a severe drawback. Artificial lighting is said to account for 90 per cent of the Mitsubishi system's operating costs.

On the other hand, there can be no doubt that research will continue in the search for breakthroughs in the development of energy-efficient lighting systems, the achievement of a higher photosynthetic ratio than in the natural environment, and in the applications of biotechnologies to factory farming.

It is to be hoped that food factory technology will not be monopolized by a group of industrialized countries and that it will be applied in those countries which need it most. ■

One of the star attractions at Expo '85 was a gigantic tomato plant, above, which produced over 13,000 tomatoes during the 6 months of the exhibition. Fed by a special nutritive solution, the plant grew in a controlled environment, with optimum lighting and temperature.

Below, inspecting tomato plants at a Japanese-made hydroponic food factory in the Maldives. The technology was adapted to conditions in this tropical developing country in the Indian Ocean.

Photo © Matsushita Electric Trading Co., Osaka

KOICHIBARA HIROSHI, *Japanese economist, is a member of the Unesco secretariat.*

Hybrids for the year 2000

by Raissa G. Butenko and Zlata B. Shamina

THE cultivation of plant tissues, cells and protoplasts has not only helped us to learn much that is new and surprising about the metabolism, genetics and capacity of the plant cell to fulfil different development programmes. It has also served as a basis for creating new technologies in agriculture and industry that are essentially different from the traditional ones. Some of these technologies are now being commercially applied, others will be used in the very near future and others in the more distant future.

Many important medicinal plants growing in natural conditions are becoming scarce, and supplies of them are limited. Consequently, their collection as medicinal raw material may lead to the complete disappearance of certain varieties. Moreover, the plantation cultivation of suitable wild plants takes up valuable space which could otherwise be sown with agricultural crops.

A solution to this problem has been found in the industrial cultivation of medicinal plant cell cultures on similar lines to cultures of micro-organism products. Using the classical methods of microbial genetics, productive stems of ginseng, a relict plant growing in a limited area of the Far East, were obtained. These stems, possessing qualities that reduce fatigue and enhance physical resistance, are being cultivated in biochemical factories. They give a high yield of biomass containing physiologically active substances. The elaboration of industrial techniques for the intensive cultivation of medicinal plant cells has proved profitable, and it is now the turn of other cell cultures such as Dioscorea and Rauwolfia.

Apart from the traditional methods of breeding micro-organisms, there are new approaches to obtaining productive stems, in particular the hybridization of partners which are actively self-propagating and possess a high degree of biosynthesis. So far, as a result of induced mutagenesis and the optimization of culture conditions, stems with a generally high level of productivity have been obtained. In some cases the content of biologically active substances was not lower than in the original plant.

Many vegetatively propagated agricultural plants accumulate pathogens, especially viruses, which it is impossible to get rid of by the usual propagation methods. But the cultivation of meristematic tips not only frees the future plant from infection but enables an unlimited quantity of offspring to be obtained from a single meristem. This technique, known as clonal micropropagation, is being widely practised all over the world for potatoes, ornamental plants and berry bushes.

Although it is much more difficult for woody species to regenerate plants from micro-shoots, conditions have been created in the Soviet Union for the mass production of poplar and aspen clones as well as those of tea plants and citrus trees. This is of great value since it makes it possible to take starting material from élite specimens and obtain within a few years entire groves of high quality trees that are completely identical to the original.

Clonal micropropagation is also extremely important for the preservation of genetic resources—both scarce, disappearing species and unique genotypes obtained through hybridization and mutagenesis. Clonal micropropagation is now becoming one of the stages of the breeding process for sugar-beet, cereal hybrids and pasture grasses.

The use of haploids in breeding is very important, especially for the selection of constant hybrids. Using traditional methods, stable forms combining useful characteristics can be obtained within ten or eleven years. The breeding of first-generation hybrid anthers shortens this process by a third or a quarter. With this method new varieties of barley, triticale, tobacco, promising original varieties of potato and many other agricultural plants have been obtained in the USSR. The new technique considerably simplifies and shortens the breeding process.

The art of obtaining so-called somaclonal variations—a broad variety of plant regenerants—is an achievement of cell biology. From amongst the somaclones it is possible to breed forms which retain all the positive characteristics of the variety but with the sought-for addition of certain necessary, especially viable traits. It is difficult to obtain such a combination of agriculturally valuable traits through the traditional breeding methods. But plants combining high productivity with resistance to fungi and viruses have been bred from potato somaclones at potato institutes in the USSR. The combination of precocity with longer grains, which it was impossible to achieve by the usual method of breeding round-grained rice plants, has proved to be possible in the rice somaclone. Somaclone variations clearly provide excellent material for breeding plants that are both productive and resistant to stress.

The cases cited above show what has been achieved by modern biotechnology. They are examples of the practical applications which have been adopted in agriculture in the Soviet Union and which have also won a place in industry. Amongst those orientations which hold out hopes of early success, that is, those which have passed laboratory tests but have not yet been widely introduced, cell breeding is the most promising. The obtaining of guaranteed harvests in zones where agriculture is exposed to risks is perhaps the most serious problem facing agriculture today. This implies the creation of strains of the principal food plants that are resistant to diseases, pests, herbicides and unfavourable environmental factors. In the Soviet Union the most important qualities are immunity to salty soils, drought and frost. Breeding at the cell level enables conditions to be created in which all cells except the resistant

Cell cultures can be a source for the manufacture of medical products derived from plants. Ginseng (Panax schinseng), below, is one plant which Soviet and Japanese scientists have cultured in this manner. Its generic name, Panax, is derived from the same Greek word as "panacea". In the East ginseng has from time immemorial been considered a cure for many ills.

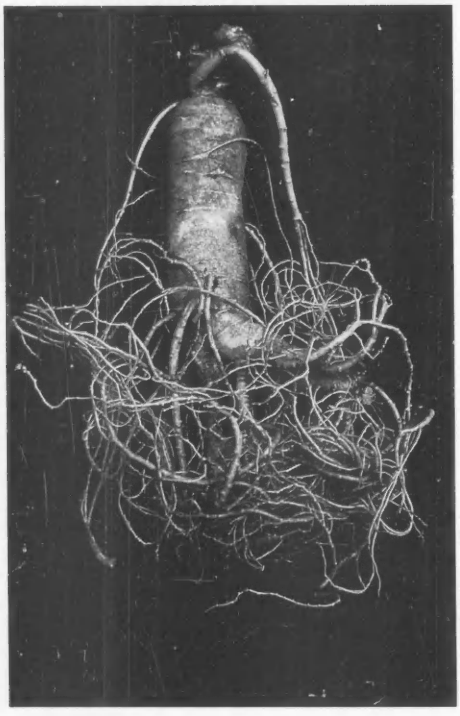
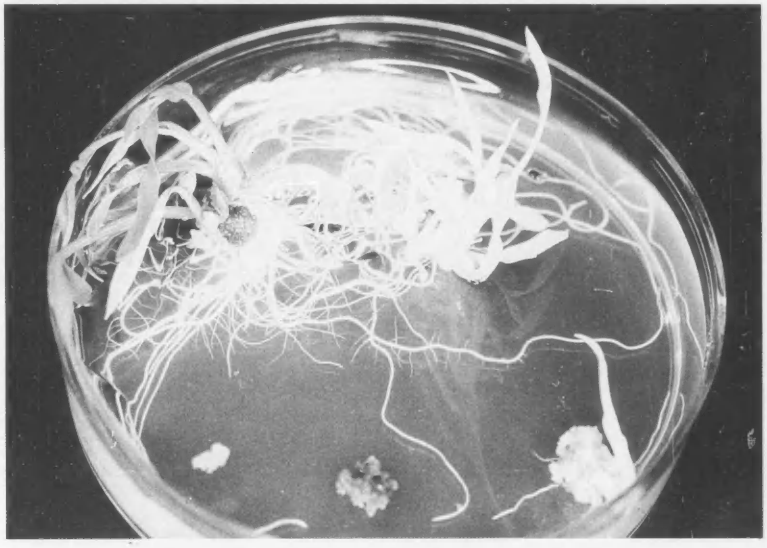


Photo M. Viard © Jacana, Paris



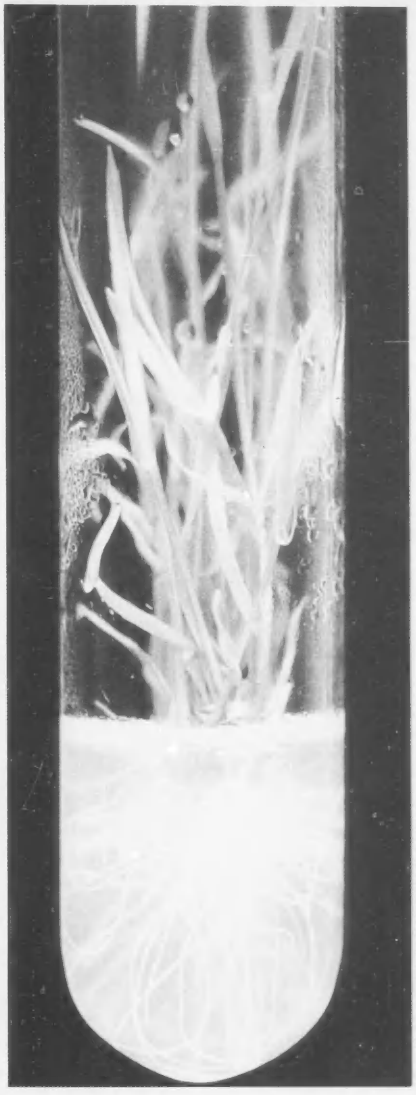


Photo C. Poisson © IRAT, Montpellier, France

The organisms in the culture dish, top, are anthers (tiny sacs of pollen). When placed in a nutrient solution they form a callus, above left, and can then be regenerated into whole haploid plants, above right. (See page 5).

ones perish and the surviving cells represent potential plants. Thus, by cultivating cells in media with a high concentration of salt, it is possible to obtain salt-resistant plants. This makes it possible, together with somaclonal variants, to conduct controlled breeding of modified cell lines and subsequently of modified plants with particularly valuable traits.

The present level and rate of development in breeding imposes a search for new methods and parent strains for creating varieties which will hold out prospects for the next century. There is already an acute need for new approaches which will ensure success later on. The way is being opened for recourse to wild varieties containing valuable genes, for the mutation of genetic material by combining somatic cells from early ancestors. There are already examples of the utilization of somatic hybrids of cultivated and wild potatoes. Thanks to a synthesis of the parents' characteristics, one of them was "endowed", through the transfer of cytoplasmic genes, with resistance to viral diseases.

The creation of "cybrids", hybrid strains which have received the cytoplasms of both parents and the nucleus of one of them, is considered to be even more promising. The possibilities for such constructions are theoretically unlimited. It is possible to transform a cell by transferring to it not only the nucleus, cytoplasm or individual organelles of the other partner, but individual genes previously cloned in bacteria.

Much of this may seem fanciful, but the fact that a broad scheme for the implementation of these processes has already been elaborated is due to the imagination of researchers. The next step will be the creation of a transformation technique. The first characteristics obtained through the transfer of individual genes will apparently be resistance to herbicides and to certain stresses and possibly a heightened biosynthetic activity if cell ferments of a biosynthesis of physiologically active substances are isolated.

The cloning of individual genes seems to be a real possibility for the near future, but the transfer of integral characteristics will be a more long-term task, for the accomplishment of which it will be necessary to learn how to isolate and clone regulatory genes. At present, the solution of this problem seems to be far-fetched and distant. But it is not so long since most of the techniques now in use in agriculture were treated as the fantasies of biologists and other scientists. ■

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ZLATA BORISOVNA SHAMINA *is a leading Soviet specialist in cell biology and plant genetics. She is the author of over 100 published scientific papers.*

For subsistence farmers in developing countries, biotechnology has much to offer. But will the potential be fulfilled?

Grains of hope

by Edward C. Wolf

FROM 1920 to 1950, agriculture in industrial countries was dominated by mechanical technologies that dramatically increased the amount of food that could be produced per worker and per hour. Shortly after the Second World War, the mechanical age gave way to the chemical age as farmers worldwide began to adopt artificial fertilizers and synthetic chemical pesticides, which vastly expanded their harvests per acre. Biotechnologies shift the focus of research toward crop plants themselves.

So far, advances have been made in industrial countries, where public scrutiny is intense. The environmental risks posed by releasing gene-spliced microbes or plants into the environment remain poorly understood. Developing regulations and guidelines for the newly emerging technologies has led to a contentious public debate about genetic engineering. In the United States, debate has centred on proposals to release bacteria modified to retard the formation of frost on strawberry and potato plants (see photo page 16). Because the bacteria could reproduce in the natural environment and thus spread beyond the fields where they were released, predicting environmental impacts is both more crucial and more complex a task than with many other technologies. Developing the "predictive ecology" that critics say is necessary for thorough environmental review, and drawing up regulations that guard against the uncertainties, will slow the marketing of commercial biotechnology products to farmers in industrial countries.

The genetic engineering of plants is far more complex than modifying microbes, but it is also less controversial on environmental grounds. Crops with modified traits are under a farmer's direct control, and their reproduction and spread in the environment are both slower and more predictable. Crop characteristics such as drought-tolerance, ability to withstand salty water, and pest resistance—the traits that have always concerned breeders—are a likely focus of the new technologies.

Given the ability to modify virtually any plant characteristic and to tailor plants in precisely defined ways, biotechnology would seem to offer tools well-suited to agricultural development strategies that emphasize resource efficiency and farming's internal resources. For example, it should eventually be possible to modify a plant's physiology to improve its efficiency in photosynthesis, enabling grains to pro-

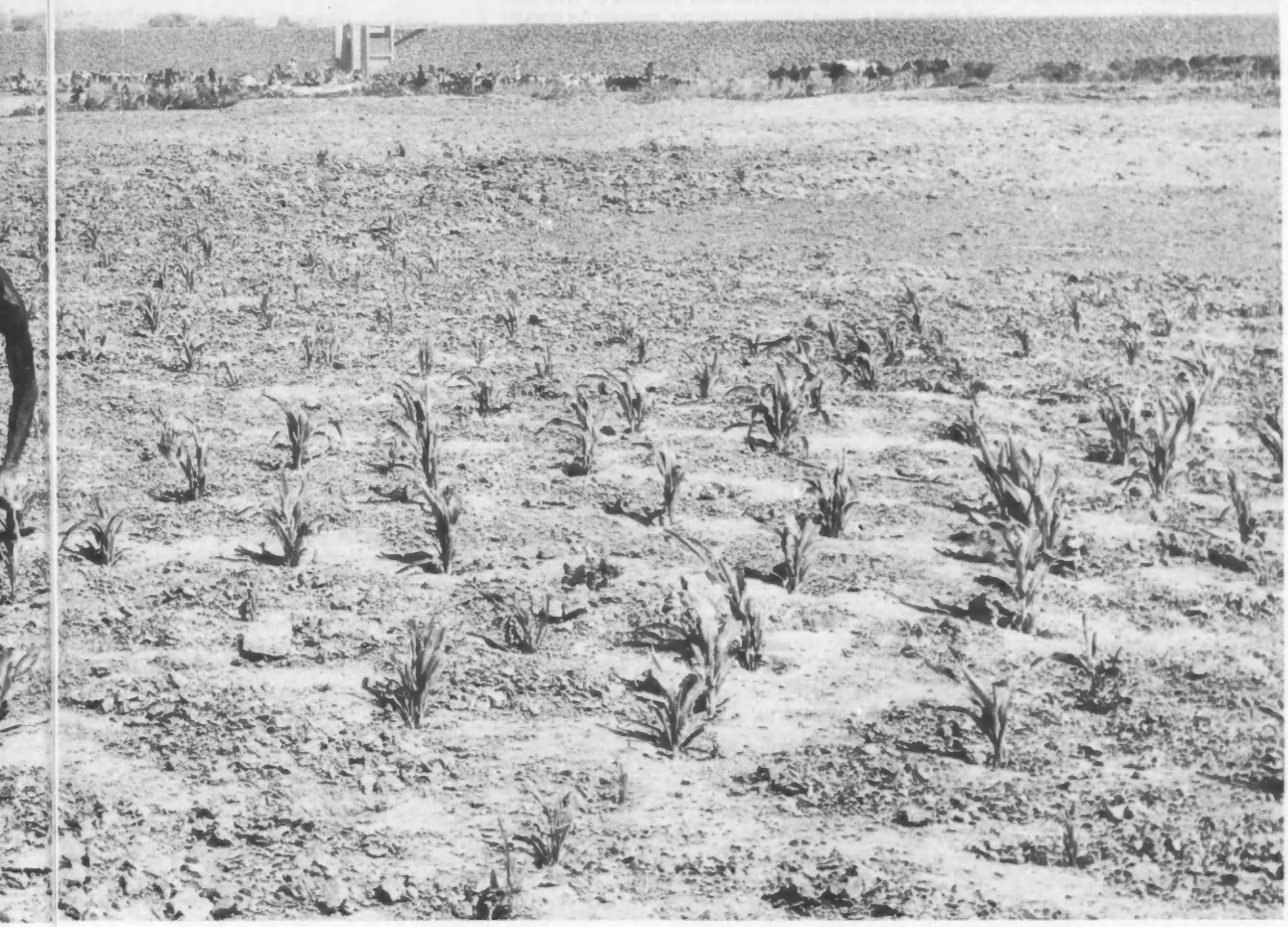


Photo © AJA Photo, Paris

duce more carbohydrate and thus higher yields. The adaptations that allow some plants to lose very little water through their leaves in transpiration, transferred to more widely grown crops, could reduce irrigation needs. Developments like these could indeed reduce pressures on marginal lands and perhaps eliminate the need for costly capital investments in water supply projects.

There is nothing in the nature of biotechnologies that renders them inherently appropriate to a strategy of efficiency and regeneration, however. Many biotechnology innovations pose trade-offs rather than clear-cut benefits. Although increasing photosynthetic efficiency could increase yields, it would also be likely to lead to accelerated depletion of soil nutrients and heavier dependence on artificial fertilizers.

The most significant factor that will affect the direction of agricultural biotechnology is the rapid shift of research from the public to the private sector. This is especially evident in the United States. For nearly a century, public agricultural experiment stations and land grant universities sponsored by the US Department of Agriculture (USDA) performed most agricultural research. Private seed companies often use the plant varieties developed by government-supported breeders. Over the last three decades, however, the private sector has assumed control of research efforts. Private companies now administer two-thirds of US agricultural research.

In biotechnology, the deck is stacked even further in favour of the private sector. USDA's Agricultural Research Service and Co-operative State Research Service support most work in agricultural biotechnology, and these two federal programmes spent less than $90 million on biotechnology research in 1984-85. Monsanto, which has the largest but by no means the only plant biotechnology research programme among private US corporations, has already invested $100 million in agricultural biotechnology development. Biotechnologies that affect agriculture in the years ahead will have a decidedly private-sector cast. With the important exceptions of mechanization and the development of hybrid corn, that has not generally been true of important innovations in agriculture.

Leaving research priorities to the marketplace may eclipse promising opportunities. Research efforts on crops will be proportional to the value of the crop and the

Farmers in developing countries growing food for their families on marginal land are vulnerable to crop failure, erratic water supply and natural catastrophes. The potential of biotechnology in allowing the rapid development of new crop varieties and hybrids that are resistant to stresses such as soil salinity and drought could be an important step towards meeting the needs of these subsistence farmers, who are largely unable to afford the costly inputs of fuel, artificial fertilizer and machinery on which past advances in agriculture have been based and whose crops have until quite recently been neglected by research. Above, hoeing millet in Mauritania.

Below, cassava (manioc) plants are protected from pests by ventilated bags as part of a biological pest-control project being carried out at the International Institute of Tropical Agriculture (IITA) at Ibadan (Nigeria). Some 200 million Africans rely on cassava for about 50 per cent of their calories. The IITA has developed disease-resistant cassava varieties for distribution in a number of African countries.

Photo G. Tartagni-FAO

size of the market. Because improving crops for small farmers in developing countries means producing low-cost agronomic innovations, many of which must be site-specific and thus not suitable for mass-marketing, crop improvement for the vast majority of the world's farmers offers little profit. Few private companies are likely to enter such an unpromising market. Consequently, investigations of minor crops like sorghum and millet, grown primarily by Third World subsistence farmers, will be neglected.

National research programmes and the international research centres have an obvious stake in applying biotechnology. Refinements in plant breeding, technologies for germplasm storage and for plant evaluation and propagation, and new alternatives in pest control, are exactly the kinds of innovations scientists need to extend research on developing-country food crops. It took decades of work to produce high-yielding varieties of wheat and rice. With biotechnology, comparable improvements in millet, sorghum, cassava, or tropical legumes could come more quickly.

The private sector domination of biotechnology raises questions about the role new technologies will play in international research programmes. Private companies may become competitors with the international agricultural research centres sponsored by the Washington-based Consultative Group on International Agricultural Research (CGIAR), particularly when it comes to improvements in major, widely traded crops like wheat and rice. The full exchange of scientific information that is essential to the international centres may be curtailed if it appears to compromise proprietary corporate research. Moreover, international centres may increasingly have to purchase or license new technologies that were formerly freely available through public channels. Finally, private firms will compete with the centres for scientific talent, and the centres may be unable to match the salaries, facilities and security that corporate laboratories offer.

Uncertainties cloud the national biotechnology programmes as well. A few developing countries, notably Indonesia, the Philippines, and Thailand, have established national programmes in agricultural biotechnology. The Philippines views its programme as the first step towards an industrialization strategy based on biological materials that can help free the country from dependence on imported oil. Philippine scientists hope to use crop residues and byproducts as raw materials to produce liquid fuels and industrial chemicals, and to develop food-processing industries with biotechnology methods. W.G. Padolina, of the National Institute of Biotechnology and Applied Microbiology at the University of the Philippines, writes, "The national strategy is to transform biomass biologically into food, fuel, fertilizers and chemicals."

Achieving these goals is certain to be costly. Few countries can afford the investment in equipment that major biotechnology programmes entail, and some countries lack sufficient numbers of trained scientists to staff such programmes. Agricultural biotechnology contrasts sharply in this regard with conventional plant breeding programmes, which require relatively modest capital investment. ■

EDWARD C. WOLF *is a Senior Researcher with Worldwatch Institute, Washington, D.C., a non-profit research organization which was created to focus attention on global problems and is funded by private foundations and United Nations organizations. The above article and the box, right, have been extracted from* Beyond the Green Revolution: New Approaches for Third World Agriculture, *a Worldwatch Paper published in late 1986.*

AGRICULTURAL scientists have recently begun to recognize that many farming systems that have persisted for millennia exemplify careful management of soil, water, and nutrients, precisely the methods required to make high-input farming practices sustainable. This overdue reappraisal stems in part from the need to use inputs more efficiently, and in part from the growing interest in biological technologies.

Traditional farming systems face real agronomic limits, and can rarely compete tonne for harvested tonne with high-input modern methods. It is important to recognize these limitations, for they determine both how traditional practices can be modified and what such practices can contribute to the effort to raise agricultural productivity.

First, most traditional crop varieties have limited genetic potential for high grain yields. They are often large-leaved and tall, for example. These traits help farmers meet non-food needs, supplying thatch, fuel and fodder as well as food to farm households. Traditional varieties respond poorly to the two elements of agronomic management that make high grain yields possible: dense planting and artificial fertilizer. Despite these limitations, traditional varieties also contain genetic diversity that is invaluable to breeders in search of genes for disease- and pest-resistance and for other traits.

Second, peasant farmers often have to plant in soils with serious nutrient deficiencies, where crop combinations and rotations are needed to help offset the limitations. Many tropical soils, for instance, lack sufficient nitrogen to sustain a robust crop. Soils in vast areas of semi-arid Africa are deficient in phosphorus. High-yielding varieties, more efficient in converting available nutrients into edible grain, can rapidly deplete soil nutrients if they are planted by peasant farmers who cannot purchase supplemental fertilizers.

Nonetheless, traditional methods can make an important contribution to efforts to raise agricultural productivity. They offer what have been called "principles of permanence". "Neither modern Western agriculture nor indigenous traditional agriculture, in their present forms, are exactly what will be needed by most small-scale farmers," says one researcher, Gerald Marten of the East-West Center in Hawaii. "The challenge for agricultural research is to improve agriculture in ways that retain the strengths of traditional agriculture while meeting the needs of changing times."

Harvest home. A Mauritanian woman fills her bowl from a heap of millet near the family tent. Research in recent years has contributed to a reappraisal of the ecological and agronomic strengths of traditional farming techniques such as those practiced by West Africa's millet and sorghum growers. Joining biotechnologies with the ecological insights of traditional farming may lead to innovative solutions to economic and environmental problems in agriculture.

Photo Naud © AAA Photo, Paris

The rediscovery of traditional agriculture

Intercropping, agroforestry, shifting cultivation, and other traditional farming methods mimic natural ecological processes, and the sustainability of many traditional practices lies in the ecological models they follow. This use of natural analogies suggests principles for the design of agricultural systems to make the most of sunlight, soil nutrients and rainfall.

Shifting cultivation practices, such as bush-fallow methods in Africa, demonstrate how farmers can harness the land's natural regeneration. Farmers using bush-fallow systems clear fields by burning off the shrubs and woody vegetation. Ashes fertilize the first crop. After a couple of seasons, as nutrients are depleted, harvests begin to decline, so farmers abandon the fields and move on to clear new land. Natural regeneration takes over; shrubs and trees gradually reseed the land, returning nutrients to the topsoil and restoring the land's inherent fertility. After fifteen to twenty years, the land can be burned and cultivated again.

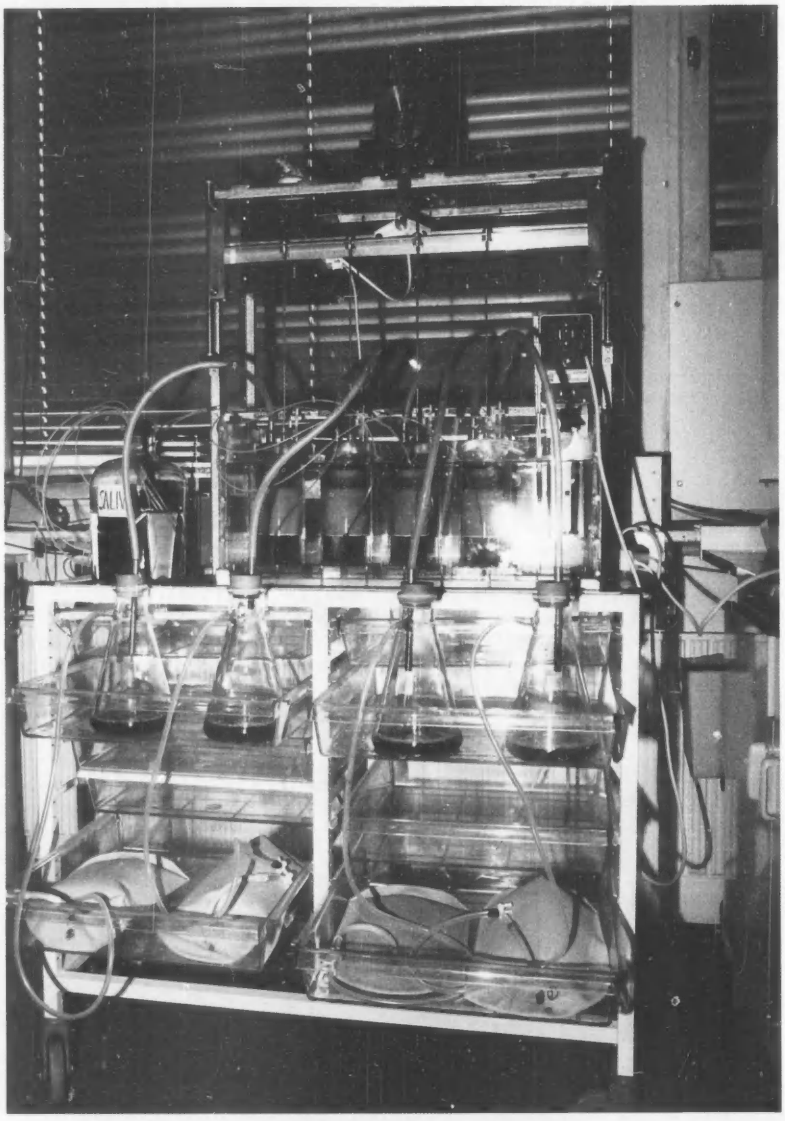
The bush-fallow system has obvious limitations. But even disintegrating systems offer a basis for designing productive and sustainable farming practices. Researchers at the Nigeria-based International Institute of Tropical Agriculture, for instance, have adapted the principles of natural regeneration in bush-fallow systems to a continuous-cultivation agroforestry system called alley cropping. Field crops are grown between rows of nitrogen-fixing trees; foliage from the trees enhances the soil organic matter, while nitrogen fixed in root nodules increases soil fertility. A high level of crop production is possible without a fallow interval. Traditional shifting cultivation provided the model for this system.

Conventional research tools can also be used to overcome the agronomic constraints that have limited traditional systems to low productivity. For decades, crop breeders have tailored varieties to respond to high levels of artificial fertilizers, assured water supplies, and dense monoculture plantings. Working with the genetic diversity available in traditional crop varieties, they can apply breeding methods to produce varieties better matched to the conditions faced by subsistence farmers.

As the African examples described here show, researchers can use traditional principles to develop new techniques that preserve the land's stability and productivity even as populations increase. Though traditional methods have limitations, they are not archaic practices to be swept aside. Traditional farming constitutes a foundation on which scientific improvements in agriculture can build. ■

Rusitec the cow

Photos FAO/IAEA

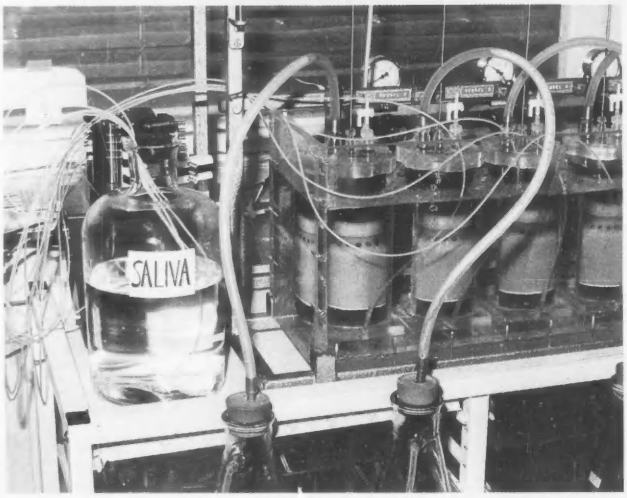


Food for rumination

The rumen is an important part of the digestive tract of ruminant animals such as cattle, buffalo, sheep and goats. It contains large numbers of micro-organisms whose function is to break down fibrous feed materials such as grass and straw and convert them to products that can be used by the animal to produce meat, milk, wool or draught power.

To study the microbial population of the rumen under controlled laboratory conditions, Dr. J.W. Czerkawski of the Hannah Research Institute, Scotland, U.K., developed an "artificial cow". The "cow", named RUSITEC (from the acronym of "Rumen Simulation Technique") is today being used as part of a project to analyse different feedstuffs being carried out by the Food and Agriculture Organization of the United Nations (FAO) and the International Atomic Energy Agency (IAEA) at their joint Agricultural Biotechnology Unit at Seibersdorff near Vienna, Austria.

In the artificial rumen micro-organisms can be indefinitely maintained by feeding a normal ruminant diet each day and providing the correct physiological conditions in terms of temperature, pH and flow of saliva. As RUSITEC chews its way through different feeds, scientists use radioactive tracing techniques to compare their digestibility. (The higher the digestibility of a foodstuff, the higher the nutritive value that can be derived from it.) By analysing the quality of different feeding materials in this way, scientists are seeking to propose improved diets for domestic animals in the developing world. Photos on this page show RUSITEC at work. Below left, the vessels representing the rumen, where microbial fermentation of diets takes place; left, the rumen simulation technique in operation; below, analysis of the end products of fermentative digestion.



Rhizobium, the farmer's Mr. Fixit

A Unesco programme to promote biotechnology for development

by Edgar J. DaSilva, J. Freire, A. Hillali and S. O. Keya

LUCERNE farmers in Kenya, bean-growers in Latin America, and rice-growers in Southeast Asia are today being helped to boost their yields as a result of a Unesco programme in which micro-organisms are being used to strengthen rural development.

The programme has been established at a time when many developing countries are facing the problem of how to increase food production to feed their expanding populations. The quality of food produced must be sufficient to provide a balanced diet with an adequate protein content. In response to this challenge, several developing nations have been expanding their agricultural lands into areas that are only marginally capable of sustaining productivity. Such attempts are invariably limited by the availability of nitrogen fertilizer, which plays a vital role in maintaining the productivity of the soil.

By the end of the third quarter of this century, world food production was dependent on a supply of synthetically produced nitrogen fertilizer amounting to 40 million tonnes and costing US $8,000-10,000 million per year. By the year 2000, estimated annual needs will be 160 million tonnes. The cost of fertilizer nitrogen, especially to the agrarian-oriented economies of the developing countries which are often beset by shortages of foreign currency, is enormous.

Nitrogen constitutes four-fifths of the earth's atmosphere and is freely available in nature. Since the nineteenth century it has been known that the roots of leguminous plants, in association with certain bacteria, are capable of extracting nitrogen from the atmosphere in the process known as nitrogen fixation. The plants concerned include groundnut, pigeon pea, mung bean, soybean, lentil, French bean, lucerne, channel clover, white clover and winged bean, to mention just a few species that are often grown in developing countries today.

The most important bacteria which are capable of nitrogen fixation belong to the genus *Rhizobium*. When the bacteria infect the plants, they stimulate the formation of nodules—swellings—on the roots. The bacteria within these root nodules improve the fertility of the soil for the host plant by adding nitrogen compounds to it. (In 1975, it was estimated that the total nitrogen fixed naturally amounted to 175 million tonnes; 35 million tonnes are fixed by cropped leguminous plants alone.)

In addition to *Rhizobium*, other micro-organisms at the heart of nitrogen-fixing systems include the *Azolla Anabaena* system which has been used for centuries by rice-growers in Asia (see *Unesco Courier*, December 1984), and the *Azotobacter*, which live independently in the soil, as well as *Spirillum* and *Clostridium*, which are associated with certain grasses (such as sugar cane) and cereal crops.

To contribute to rural development by promoting biological nitrogen fixation techniques in developing countries, Unesco has established, with support from the United Nations Environment Programme (UNEP) and the Food and Agriculture Organization of the United Nations (FAO), a number of Microbiological Resources Centres (MIRCENs) in different parts of the world to catalogue and preserve *Rhizobium* and other micro-organisms of economic significance and to train local manpower in their use. The Centres form part of a global network of MIRCENs which are concerned with the application to development of a whole range of biotechnological applications, from the production of biogas to biopharmaceuticals.

By inoculating seeds with cultures of an appropriate *Rhizobium* species, or cyanobacteria in the case of rice, it is possible to increase the supply of nitrogen available to the plants concerned. The production and use of such biofertilizers can thus greatly help both to increase the productivity levels of the planet's soil resource and to conserve petroleum and its expensive technologically-processed products, such as fertilizer.

In interaction with other international programmes, schemes focused on the development of biofertilizers or *Rhizobium* inoculant material are already operating through the MIRCEN network on a level of regional co-operation in Latin America, East Africa and Southeast Asia and the Pacific, with additional support from FAO and UNEP.

In the field of biological nitrogen fixation

Below, bilingual (English-Swahili) instructions for farmers on a packet of fertilizer produced from bacteria by a Unesco-supported Microbiological Resources Centre (MIRCEN) in Kenya. The fertilizer, mixed with the seeds of certain leguminous plants such as beans or clover at sowing time, helps the plants to increase their intake of nitrogen from the atmosphere which is essential for their growth.

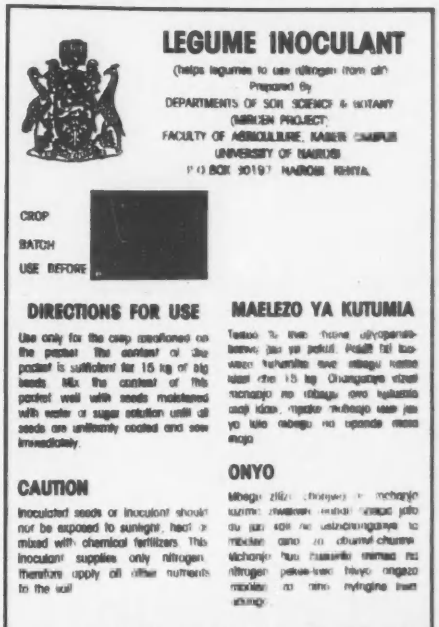

LEGUME INOCULANT
DEPARTMENTS OF SOIL SCIENCE & BOTANY
(MIRCEN PROJECT)
FACULTY OF AGRICULTURE, KABETE CAMPUS
UNIVERSITY OF NAIROBI
P.O.BOX 30197 NAIROBI KENYA
CROP
BATCH
USE BEFORE
DIRECTIONS FOR USE
Use only for the crop mentioned on the packet. The content of the packet is sufficient for 15 kg of big seeds. Mix the content of this packet well with seeds moistened with water or sugar solution until all seeds are uniformly coated and sow immediately.
MAELEZO YA KUTUMIA
CAUTION
Inoculated seeds or inoculant should not be exposed to sunlight, heat or mixed with chemical fertilizers. This inoculant supplies only nitrogen, therefore apply all other nutrients to the soil
ONYO
STORE THIS PACKET UNDER COOL PLACE UNTIL USE
WEKA PAKITI HII PAHALI PASIPO JOTO AU JUA KALI

Photo Michel Claude/Unesco

UNESCO MIRCEN

international co-operation

Aim: Have young scientists contribute to research and development in their own countries and promote international co-operation by working with high-level scientists in UNESCO training courses.

Mechanisms

1 MIRCENs

MIRCENs= Microbiological Resources Centres

A world-wide network

Aim: Collect, preserve and use microbial strains for national development and international co-operation

2 GIAM

GIAM = Meetings of Scientists on Global Impacts of Applied Microbiology

Aim: Strengthen research co-operation between industrial and developing countries
Stimulate capacity-building for local research and training

3 Co-operation with U.N. Agencies and non-governmental organizations

FAO Food and Agriculture Organization
UNDP United Nations Development Programme
UNEP United Nations Environment Programme
UNIDO United Nations Industrial Development Organization
ICRO* International Cell Research Organization
IFS International Foundation for Science
IOBB International Organization of Biotechnology and Bioengineering
IUMS International Union of Microbiological Societies
WFCC World Federation for Culture Collections

* Panel on Microbiology and Biotechnology

4 Co-operation with regional organizations

ALAR Latin-American Association of Rhizobiology
AABNF African Association for Biological Nitrogen Fixation
ABEGS Arab Bureau of Education for the Gulf States
CEC Commission of the European Communities
SANEM Southeast Asian Network on Microbiology

five MIRCENs are already operating, in Kenya, Brazil, Hawaii, Beltsville, USA, and Senegal. The broad responsibilities of the MIRCENs for the East African and West African regions include the collection, identification, maintenance, testing and distribution of rhizobial cultures compatible with local crops. Deployment of local rhizobia inoculant technology and promotion of allied research are other activities. Advice and guidance are provided regionally to individuals and institutions engaged in rhizobiology research.

The MIRCEN in Latin America, in a similar vein, promotes the identification of leguminous germplasm of high symbiotic capacity and soil-limiting factors; the optimal selection of efficient rhizobial strains for soybean, clover, lucerne, lotus, peas, beans and the cowpea group; optimization of inoculant production for use in demonstration plots and by small farmers; and the quality control of inoculants for use by private and official laboratories.

The goals of the MIRCENs at Hawaii and Beltsville, U.S.A., are to contribute towards alleviating the dependence of developing countries on chemically-derived nitrogenous fertilizers. This is accomplished through research to provide a database to assess the benefits of utilization of legume-based biological nitrogen fixation (BNF) technologies; development and delivery of validated BNF technologies that are appropriate to the needs of, and circumstances in, developing countries; and the provision of support services.

In addition, the MIRCENs in Kenya and Brazil are involved in the production of inoculants for legume trials and for use by farmers.

Inoculant production at the East African MIRCEN has now been initiated using strains tested by MIRCEN workers. Inoculants for eleven pasture legumes have been supplied to FAO pasture agronomists in Kenya. The MIRCEN in Brazil, on behalf of the Federal Government, is responsible for the quality control of inoculants produced in that country. An average of 100 samples are examined per year. On a limited basis this service is also available to institutions in the developing countries. The BNF MIRCENs play a valuable role in maintaining and distributing efficient cultures of *Rhizobium*. Collectively, over 3,000 strains are maintained in the MIRCEN collections. ■

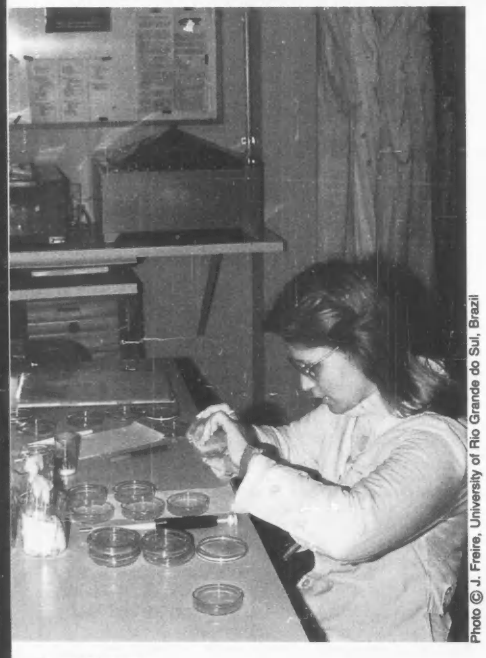


Photo © J. Freire, University of Rio Grande do Sul, Brazil

A technician at a Unesco-supported Microbiological Resources Centre (MIRCEN) in Brazil prepares materials for a training course in the identification of bacteria from which fertilizer can be produced.

EDGAR J. DASILVA, *Indian microbiologist, is a former Vice-President of the World Federation of Culture Collections (WFCC). The author of several papers on the biotechnological applications of micro-organisms, he is a member of Unesco's Division of Scientific Research and Higher Education, where he has been largely responsible for the development and implementation of Unesco's MIRCEN programme.* **J. FREIRE,** *of Brazil, is director of the MIRCEN and a professor of soil microbiology at the Universidade Federale do Rio Grande do Sul.* **A. HILLALI,** *of Morocco, is a rhizobiologist at the Institut Agronomique et Vetérinaire Hassan II, at Rabat. He has been carrying out research and consultancy work on the applications of rhizobial biofertilizers at the West African MIRCEN at Bambey, Senegal.* **S.O. KEYA,** *of Kenya, is director of the MIRCEN at the University of Nairobi, where he is head of the department of soil science and dean of the faculty of agriculture.*

Photo Y. Duval © ORSTOM, Paris

A challenge for the developing world

by Albert Sasson

In a Kuala Lumpur laboratory, above, Malaysian biotechnicians tend test-tube cultures as part of a short-term project to produce elite oil-palms.

BIOTECHNOLOGIES have much to offer developing countries. Their application to agriculture, horticulture and forestry can contribute largely both to the improvement of cultivated plants and to the protection of species threatened with disappearance. But careful consideration must be given to the choice of the most appropriate techniques and their transfer and adaptation to specific conditions.

Such processes as plant cell and tissue cultures and genetic engineering are tools, not solutions to social problems. For example, the replacement of traditional crop varieties by new ones may cause unemployment if such new varieties require less work. There is also a tendency for research in biotechnology to respond primarily to the needs of international markets rather than the domestic needs of developing countries. Furthermore, since it is the big landowners who possess the financial and management resources enabling them to profit from technological innovations, it is likely that they rather than the small farmers will gain from the application of biotechnology to agriculture.

There is a strong possibility that the poor countries in general will not only reap few direct benefits from the biotechnological revolution, but that their economies will be indirectly hit by the development of new products (such as artificial sweeteners) which will compete with their traditional export commodities. There is a danger that the technology gap between the rich and poor countries will grow even wider. Searching questions must therefore be asked about the nature of the "Biotechnological Revolution" and its long-term economic, social and geopolitical effects; strategies must be devised to ensure that its benefits are equitably shared both between countries and between different social groups within a given country.

The "Biotechnological Revolution" is irreversible, if only because of the commercial successes that have already been achieved and the size of the investments which have been made in the different fields of biotechnology. It has been estimated that the sales of products derived from the application of biotechnologies to food and agriculture alone may reach between $50,000 million and $100,000 million by the year 2000.

Whereas the "Green Revolution" was largely carried out by the public sector, which made possible the free exchange of new plant strains developed notably in international agronomic research centres sponsored by the FAO, the impetus behind the "Biotechnological Revolution" in agriculture is coming largely from the private sector, although much basic research is being carried out in universities and in state-supported agricultural and forestry facilities.

The privatization of the results of research in biotechnology means that these results do not form part of the universally available body of scientific and technical knowledge that belongs to the common heritage of mankind. In addition, the public sector research institutions and the organisms which subsidize them are tending to

The Green Revolution and the "Biorevolution"

Characteristics	Green revolution	Biorevolution
Crops affected	Wheat, rice, maize	Potentially all crops, including vegetables, fruits, agro-export crops (e.g., oil palms, cacao), and speciality crops (e.g., spices, scents)
Other products affected	None	Animal products, pharmaceuticals, processed foods, energy
Areas affected	Some locations in some less developed countries (i.e., if accompanied by irrigation, high-quality land, transport availability, etc.)	All areas, including marginal lands characterized by drought, salinity, aluminium toxicity, etc.
Technology development and dissemination	Largely public or quasi-public sector	Largely private sector (multinational corporations and start-up firms).
Proprietary consideration	Patents and plant variety protection generally not relevant	Processes and products patentable and protectable
Capital costs of research	Low	High
Research skills required	Conventional plant breeding and parallel agricultural sciences	Molecular and cell biology expertise plus conventional plant-breeding skills
Crops displaced	None (except the germplasm resources represented in traditional varieties)	Potentially any

Source: *Ceres*, FAO / Buttel et al

take out patents and thus contribute to the privatization of the results of research.

The growing tendency to grant patents to plant breeders as a means of protecting the creation of new strains is causing widespread concern in developing countries where these measures are seen as obstacles to their efforts to increase their agricultural output.

The adoption of legislation to protect the rights of plant breeders by patent (that is, granting to plant geneticists and the bodies which subsidize their research exclusive production and marketing rights over these varieties) has encouraged multinational corporations and several major national chemical and pharmaceutical groups to buy seed marketing companies and to take majority participations in firms engaged in varietal selection and plant genetics research. In Europe and North America, the leading multinational petrochemical and pharmaceutical firms have acquired a dominant position in this field.

One reason for this trend is to be found in the complementary roles played by seeds, fertilizer, pesticides and animal medicines in boosting agricultural output. It is thus possible for one firm to influence the entire production chain.

The market for selected seeds is worth $12,000 million a year, including $2,000 million for hybrid maize and sorghum seeds, and $1,000 million for hybrid oats, soya and cotton seeds. The grip of the multinational corporations and of other major industrial groups on the seed companies is likely to encourage monopolies and sharply reduce the public sector role in plant selection.

Measures taken by the technologically advanced countries to protect the results of increasingly expensive research into plant genetics and to ensure that such research is profitable include the payment of royalties, notably by the developing countries, for seed varieties selected in the industrialized countries. The latter countries are also tending to use their collections of seeds and plants (germplasm) for commercial purposes; the private sector is playing an increasing role in the collection, preservation and use of germplasm.

Many developing countries do not possess the financial and technical resources to establish seed collections or to preserve such collections in satisfactory conditions. They have no alternative but to buy new strains selected from varieties which have been cultivated or which grow wild in their own regions. Such varieties may have been domesticated, cultivated and improved by many generations of farmers in the developing countries before being crossed with other varieties, protected by patent, and then sold in their countries of origin as "new and different". This paradoxical situation calls in question the validity of the patent system, for to grant ownership rights and royalties to those who have recently improved the genetic heritage of a plant variety is to disregard the efforts of all those who have previously transformed the variety and derived no profit from it.

"The North may be 'grain-rich' but the South is 'gene-rich':" the genetic resources of most cultivated plants are found in the developing countries, notably in the tropics, but the selection and improvement operations relating to these plants mainly take place in the industrialized countries. In 1982, according to the Organization for Economic Co-operation and Development (OECD), the developing countries were contributing an estimated $500 million a year to the United States wheat harvest. This contribution resulted from the use of plant genetic resources which originate in the developing countries and are indispensable to the improvement of cultivated plants and variety selection in the United States and in the other industrialized countries.

In view of the economic importance of their plant genetic resources, the developing countries intend to protect these resources—for example by preventing the export of plant reproducing material. They also feel that the purchase price of varieties of seeds selected and improved from their own phytogenetic stock is excessive and that it is unjust to be thus obliged to buy back indirectly a part of their phytogenetic heritage.

The risk of a diminution of genetic diver-

Photo UNEP/De Silva

Completing the brick dome of a digestion tank for biogas production in China. With over 7 million biogas digesters, China leads the world in this field of energy production.

sity, combined with the question of restrictive practices in the distribution of material needed for the improvement of cultivated plants, has led to a search for an international agreement on the conservation of plant genetic resources, considered as part of the heritage of humanity, and on their equitable use, instead of allowing such use to be regulated solely by national jurisdictions. If the industrialized countries wish to have access to the plant genetic resources of the developing countries and wish to use the hardy local strains which are found there, the developing countries wish to benefit from services provided by gene banks in the industrialized countries and to claim their national sovereignty over plants grown in their countries.

The problems of the conservation of and free access to plant genetic resources have thus assumed a geopolitical dimension in the context of the debate on the exploitation of the Earth's resources for the benefit of all humanity. In November 1981 a resolution presented by Mexico to the 21st Conference of FAO invited the Director-General to prepare a draft international Convention on the conservation of plant genetic resources necessary to increase agricultural production, on the removal of obstacles to the free distribution of plant material and on the improvement of international co-operation in this field.

In November 1983, a draft international Convention was accordingly submitted for examination to the FAO Conference at its 22nd Session. Among its provisions was one which prohibited the imposition of any restriction on the availability or exchange of plant genetic resources for agriculture and food production.

The 156 countries represented at the 22nd Session recognized that "plant resources were part of the common heritage of mankind and should thus be accessible without restriction". Such resources include wild species or those close to cultivated varieties, which should be catalogued and protected, for they are threatened with disappearance, as well as the most recent cultivated varieties and strains which make it possible to produce seeds of more productive hybrid varieties.

In November 1985, at the 23rd Session of the FAO Conference, the industrialized countries opposed the creation of an international mechanism to ensure the free exchange of plant genetic resources and to abolish payment for varieties selected in the industrialized countries and royalties to acquire them. It is thus to be expected that genetic information needed for the improvement of cultivated plants may become a commercial commodity subject to competition between seed companies, between countries, and between seed companies and countries.

However, it is to be hoped that a compromise will be found between the legitimate desire to reward human ingenuity by granting patents to selectors in the industrialized countries and the need for developing countries to obtain selected varieties at a price compatible with their limited means and the imperatives of their agricultural development. It would also be ethically justifiable to take into account, in the sale to developing countries of seed varieties selected from their own cultivated plants, the work of the generations of farmers who have contributed to the improvement of these plants.

In the meantime, much can be done to harness biotechnologies to agriculture, horticulture and forestry in the developing world.

First of all, in each developing country steps should be taken to establish priorities and economic objectives which derive maximum benefit from the available resources. Those biotechnological processes which are most relevant to the country's social and economic needs should be identified and inventories of local resources should be drawn up.

Secondly, developing countries should avoid entering into competition, at least ini-

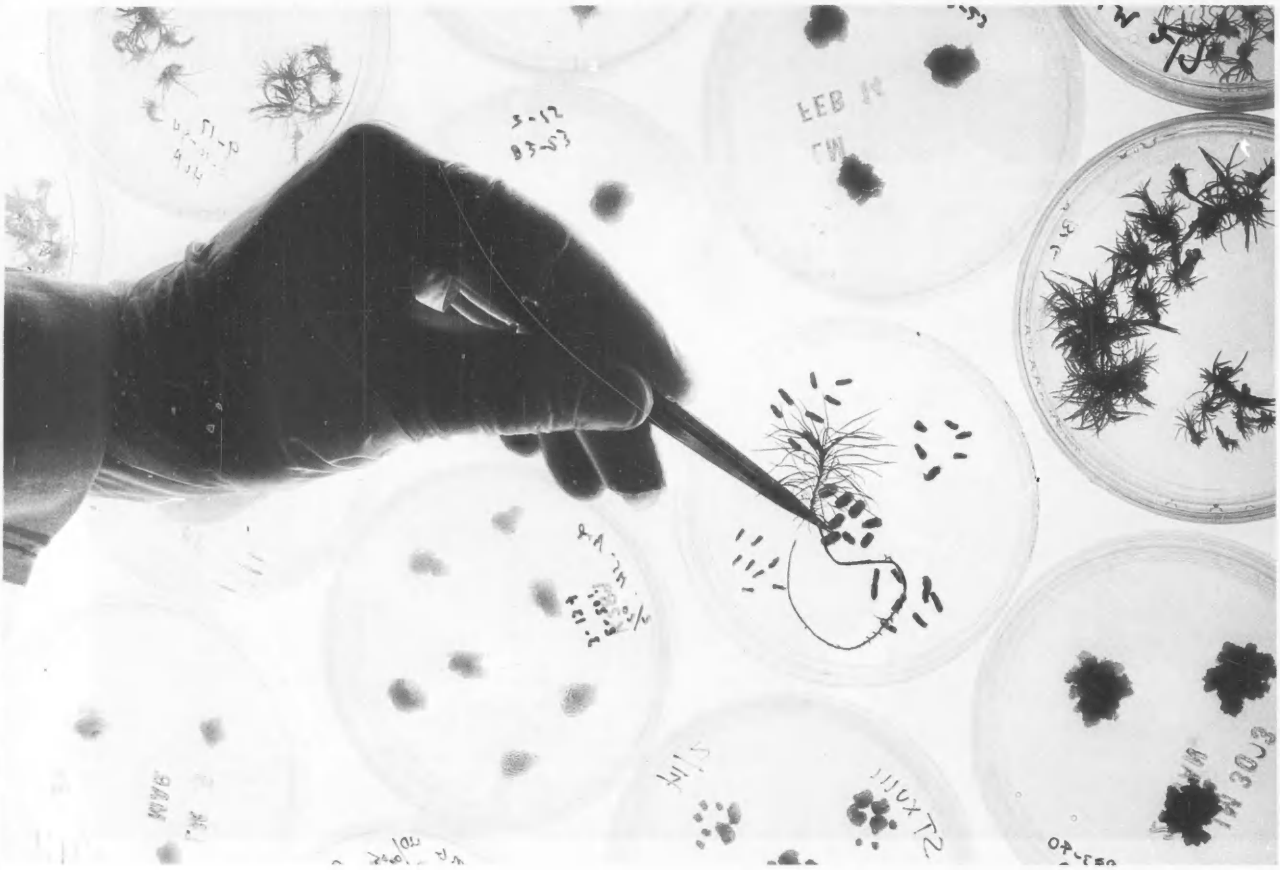


Photo © Brownie Harris, New York

From test-tube to forest

Biotechnology opens new possibilities for forest management in developing countries. In vitro tissue culture methods, above, make possible the rapid production of genetically uniform elite specimens which must then be tested in field conditions in the outdoor laboratory—the forest. Lines of eucalyptus in the Congo, right, stretch as far as the eye can see. They were grown from clones of carefully selected hybrids.

tially, with the industrialized countries in such advanced fields as gene transfer and genetic engineering. They should profit from simpler techniques of plant tissue culture, meristem culture (see page 6) and plant organ culture for the rapid vegetative propagation of the most useful strains and for the isolation of virus-free strains. They should adopt and practise at the appropriate scale low-cost, proven biotechnologies which are easy to transfer and to adapt to local conditions.

Thirdly, biotechnologies should not be considered as the only means of improving species of cultivated plants. They should be seen as complementary to hybridization methods and efficient agricultural practices. The success of biotechnologies in the developing countries will depend to a large extent on their being closely associated with classical methods of crossing and improving plants, with agricultural training programmes, the establishment of remunerative farm prices and the existence of a good marketing network for agro-food products.

Fourthly, the choice of appropriate biotechnologies does not mean that we should resign ourselves to accepting an international division of biotechnologies: high tech for the technologically advanced countries, outworn technologies for the developing countries. A range of biotechnologies, of varying degrees of sophistication and complexity, should exist in each specific situation. Every national scientific and technological community should take into account international developments in biotechnology when considering local needs, and be able to use the most advanced techniques or adapt them to development projects.

Whatever options for the development of biotechnologies are chosen, education and training are bound to play an essential role. Standing as they do at the crossroads of several disciplines of the life sciences (genetics, biochemistry, physiology and microbiology), and of engineering (fermentation technology, automization of production techniques, chemical and industrial microbiology), biotechnologies call for interdisciplinary training programmes and an integrated approach.

There is a chronic lack of specialists and biotechnology technicians in the developing countries. According to one survey, in 1983 there were 23,000 researchers in this field in the USA, 12,000 in the USSR, 8,000 in Japan, 3,400 in the rest of Asia, 1,900 in Latin America and 400 in Africa.

International and regional co-operation undoubtedly has a major role to play in encouraging the transfer of biotechnologies and the fulfilment of their promise in the developing world, as well as helping to solve the ethical problems involved. It should be

possible for countries within a given region to carry out joint research projects into matters of common concern and to obtain results which can be applied in several countries. Efforts must be made to encourage co-operation between developing and industrialized countries, including private sector institutions in these countries. Examples of such co-operation already exist. They include the production of vaccines against foot and mouth disease in Botswana with the co-operation of Rhône-Mérieux in France; biogas production from wastes through co-operation between India, China and several developing countries; and the cloning of the oil palm and the creation of new oil palm plantations in Ivory Coast, Malaysia and Indonesia in co-operation with the French Office of Scientific and Technical Research Overseas (ORSTOM) and the French Research Institute for Oils and Oil-Producing Substances (IRHO).

The role of international intergovernmental organizations is important in helping to provide governments with consultative services with a view to the formulation of national policies and programmes in biotechnology; to encourage joint research projects and other joint activities between developing and industrialized countries; to encourage researchers and technicians from all countries to take part in these activities, and to strengthen national research and training capacities.

Ever since its early days, Unesco has laid great stress in its scientific programmes on international co-operation in research and training in the life sciences, and at an early stage drew attention to the importance of research into micro-organisms and embarked on a programme in applied microbiology. In 1962 Unesco sponsored the creation of the International Cell Research Organization (ICRO), and in 1972 joined with ICRO and the United Nations Environment Programme (UNEP) in launching a world programme to safeguard the genetic heritage of microbial resources and to make this heritage accessible to developing countries. Then, in 1975, Unesco began to create the world network of Microbiological Resources Centres (MIRCEN) whose activities are described in the article on page 27.

Following the adoption of Unesco's second Medium-Term Plan (1984-1989), activities relating to training, research and international co-operation in applied microbiology were strengthened and further extended into the field of biotechnologies. Today, in close collaboration with FAO, the United Nations Industrial Development Organization (UNIDO), the World Health Organization (WHO) and other specialized institutions of the United Nations system and with international non-governmental organizations, Unesco continues this work as part of the broader effort to enable the developing countries to contribute to and benefit from advances in scientific knowledge. ■

ALBERT SASSON, *Moroccan microbiologist, is a doctor of natural sciences of the University of Paris. From 1954 to 1973 he was engaged in research at the Rabat (Morocco) Faculty of Sciences into algology, the microflora of arid lands, and free and symbiotic nitrogen-fixing micro-organisms. A member of the Unesco secretariat since 1974, Dr. Sasson is the author of several books and studies on biology, microbiology and biotechnologies and their applications to development, notably* Biotechnologies: challenges and promises *(Unesco, 1984), which has so far been published in English, French, Spanish, Italian, Chinese and Russian and will soon be appearing in Bulgarian, Portuguese and Romanian. In preparing this issue, the Editors have made extensive use of Dr. Sasson's book* Quelles biotechnologies pour les pays en développement ? *(Unesco and Biofutur publishers, Paris, 1986), from which the above article has been adapted. Dr. Sasson's latest book is* Nourrir demain les hommes, *("Feeding mankind tomorrow") published by Unesco, 1986.*

Photo © Brownie Harris, New York

Photo © Periscoop, Paris

Glossary

Aerobic. Living or acting only in the presence of oxygen.

Allogamy. Cross-fertilization in flowering plants.

Amylolytic. Capable of converting starch into sugar.

Anaerobic. Living or acting only in the absence of oxygen.

Autogamy. Self-fertilization in flowering plants.

Biomass. All organic matter that grows by the photosynthetic conversion of solar energy.

Biotechnology. Techniques that use living organisms or substances from those organisms, to make or modify a product, and including techniques used for the improvement of the characteristics of economically important plants and animals and for the development of micro-organisms to act on the environment.

Callus. An undifferentiated cluster of plant cells that is a first step in regeneration of plants from tissue culture.

Cell. The smallest structural unit of living matter capable of functioning independently; a microscopic mass of protoplasm surrounded by a semi-permeable membrane, usually including one or more nuclei and various non-living products, capable, either alone or by interacting with other cells, of performing all the fundamental functions of life.

Cell culture. The *in vitro* growth of cells isolated from multi-cellular organisms. These cells are usually of one type.

Cell fusion. Formation of a single hybrid cell with nuclei and cytoplasm from different cells.

Chromosomes. The rodlike structures of a cell's nucleus that store and transmit genetic information; the physical structure that contains genes. Chromosomes are composed mostly of DNA and protein and contain most of the cell's DNA. Each species has a characteristic number of chromosomes.

Clone. A group of genetically identical cells or organisms produced asexually from a common ancestor.

Culture medium. Any nutrient system for the artificial cultivation of bacteria or other cells; usually a complex mixture of organic and inorganic materials.

Cytoplasm. The "liquid" portion of a cell outside and surrounding the nucleus.

Deoxyribonucleic acid (DNA). The genetic material found in all living organisms. Every inherited characteristic has its origin somewhere in the code of each individual's DNA.

Fermentation. An anaerobic bioprocess. Fermentation is used in various industrial processes for the manufacture of products such as alcohols, acids and cheese by the action of yeasts, moulds and bacteria.

Gene. The basic unit of heredity; an ordered sequence of nucleotide bases, comprising a segment of DNA.

Genome. The genetic endowment of an organism or individual.

Genotype. The genetic makeup of a given organism.

Germ cell. The male and female reproductive cells; egg and sperm.

Germplasm. The total genetic variability available to a species.

Hybrid. The offspring of genetically dissimilar parents (e.g. a new variety of plant or animal that results from cross-breeding two different existing varieties, a cell derived from two different cultured cell lines that have fused).

In vitro. Literally, *in glass*; pertaining to a biological reaction taking place in an artificial apparatus; sometimes used to include the growth of cells from multicellular organisms under cell culture conditions.

Meristem. The undifferentiated plant tissue from which new cells arise.

Metabolism. The physical and chemical processes by which foodstuffs are synthesized into complex elements, complex substances are transformed into simple ones and energy is made available for use by an organism.

Molecule. A group of atoms held together by chemical forces; the smallest unit of matter which can exist by itself and retain its chemical identity.

Mutagenesis. The induction of mutation in the genetic material of an organism; researchers may use physical or chemical means to cause mutations that improve the production capabilities of organisms.

Nitrogen fixation. The conversion of atmospheric nitrogen gas to a chemically combined form, ammonia (NH_3) which is essential to growth. Only a limited number of micro-organisms can fix nitrogen.

Nodule. The anatomical part of a plant root in which nitrogen-fixing bacteria are maintained in a symbiotic relationship with the plant.

Nucleus. A relatively large spherical body inside a cell that contains the chromosomes.

Pathogen. A disease-producing agent, usually restricted to a living agent such as a bacterium or virus.

Protoplast fusion. The joining of two cells in the laboratory.

Recombinant DNA. The hybrid DNA produced by joining together *in vitro* pieces of DNA from different organisms.

Somaclonal variation. Genetic variation produced from the culture of plant cells from a pure breeding strain; the source of variation is not known.

Species. A taxonomic subdivision of genus. A group of closely related, morphologically similar individuals which actually or potentially interbreed.

Totipotency. The capacity of a higher organism cell to differentiate into an[illegible]re organism. A totipotent cell contains all the[illegible]etic information necessary for complete develo[illegible]ment.

Vector. DNA molecule used to introduce foreign DNA into host cells. Vectors include plasmids, bacteriophages (viruses), and other forms of DNA. A vector must be capable of replicating autonomously and must have cloning sites for the introduction of foreign DNA.

Vegetative propagation. Reproduction of plants other than by seeds; that is, division of part of the plant body. New plants are genetically identical to parent plant.

Definitions included in this section have been largely taken from a glossary published in ATAS Bulletin (1, Nov. 1984), a publication prepared by the Centre for Science and Technology for Development, United Nations, New York

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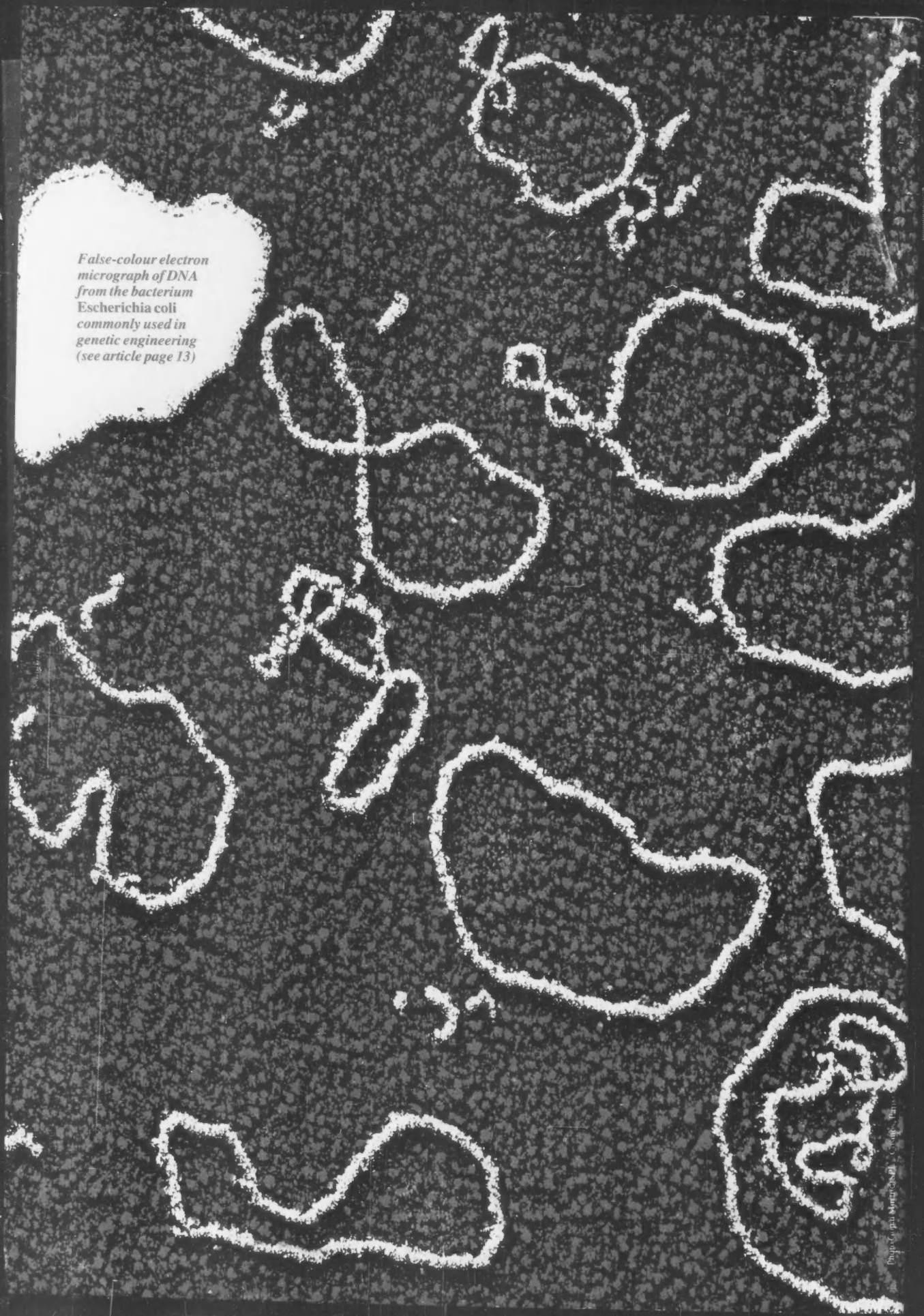

False-colour electron micrograph of DNA from the bacterium **Escherichia coli** *commonly used in genetic engineering* *(see article page 13)*